THE CHEMISTRY OF HUMAN COLON COLLAGEN

LINDA WESS

THESIS SUBMITTED FOR THE DEGREE OF Ph.D.

UNIVERSITY OF EDINBURGH

1992

DECLARATION

The work presented in this thesis was carried out between October 1986 and October 1989, under the supervision of Dr. Martin A. Eastwood at the Western General Hospital, Edinburgh, and Professor Andrew Miller at the Department of Biochemistry, University of Edinburgh. All material presented in this thesis, unless otherwise stated, is the sole work of the author, as is the composition.

ABSTRACT

This thesis describes the study of collagen from the human colonic wall in healthy subjects and in those subjects with colonic diverticulosis, in relation to aging of the tissue. This thesis also examines the effect of diet on the collagen of the colon of the laboratory rat, as a model for the study of the human subject.

Chapters 1 and 2 describe the background to the study. Chapter 1 describes the structure and function of the human colon and some of the conditions which affect it. Chapter 2 describes the structure and previous studies on connective tissues and the relationship between structure and function.

Chapter 3 describes the materials and methodology used in the work in this thesis, for examining, both the effect of age and diet on the collagen of the colon. This involves biochemical and structural analysis.

Chapter 4 presents data from the study of the effect of aging on the collagen from healthy colons and those with colonic diverticulosis. The chapter describes biochemical and structural evidence for altered colonic collagen in the aged tissue.

Chapter 5 presents data from the study of the effect of diet on colonic collagen using the laboratory rat as a suitable model for the situation in humans. This chapter describes biochemical and structural evidence for altered colonic collagen as a consequence of a diet low in dietary fibre. This chapter also examines the effect of maternal diet on the health of the offspring. The chapter also describes the possible role of dietary fibre in protection against colonic diverticulosis.

Chapter 6 discusses the techniques used and the results produced. The chapter discusses the two theories relevant to the aetiology of colonic diverticulosis, the way in which these theories conflict and run in parallel. The inevitability of colonic diverticula as a consequence of age is discussed. A possible mechanism for the development of colonic diverticulosis is described. The techniques and their potential in further studies is discussed.

ACKNOWLEDGEMENTS

I gratefully acknowledge the financial support provided by the Faculty of Medicine Scholarship Fund, for research during my two years at the Western General Hospital, Edinburgh, and my year at the Department of Biochemistry, University of Edinburgh.

I would like to express my gratitude to the following friends and colleagues who have helped me in the work for this thesis:

Dr. Martin Eastwood for his supervision, unfailing support and advice throughout this work. Professor Andrew Miller and Dr. Tim Wess for expert supervision and advice on the X-ray diffraction work.

Professor Anthony Busuttil and the staff at the Western General Hospital Mortuary for tissue collection, *post -mortem* examination of tissues, and their kind advice.

Keith Chalmers, Andrew Wilson and all other staff from the Animal Unit at the Western General Hospital, for their expertise in animal care.

Dr. Colin Jackson and Mr. Wim Bras for assistance with the fibre diffraction cameras at the Daresbury Sychrotron Radiation Source.

Medical illustration for the production of the plates incorporated in this thesis.

All my friends, past and present from the Wolfson Gastrointestinal Laboratories and from room 302 who have provided many fruitful discussions and a lot of laughter.

Finally I would like to thank my Mum, my sisters Lesley and Gillian and my husband Tim for their love and unfailing support throughout this project.

List of Abbreviations and Symbols.

Å :	Angstroms.
Asc. :	Ascending colon.
A.F.G.P.:	1-alkyl-2-formyl-3,4-diglycosyl-pyrrole.
cm :	Centimetres.
D.D.:	Diverticular disease.
Desc. :	Descending colon.
g :	Grammes.
F.F.I.:	2-(-2furoyl)-4-(-5)-(2-furanyl)-1-H-imidazole.
F.H.F.:	Familial high fibre fed group.
F.L.F. :	Familial low fibre fed group.
HHMD:	Histidinohydroxymerodesmosine.
HLNL:	Dehydrohydroxylysinonorleucine.
HLOLN:	Hydroxylysino-5-ketonorleucine.
H.P.:	Hydroxylysyl pyridinoline.
L.P.:	Lysyl pyridinoline.
m :	Metres.
mm :	Millimetres.
N.C.D.:	Non crystalline diffraction.
N.S.:	Non significant.
S.E.M.:	Scanning electron microscopy.
S.E.R.C. :	Science and Engineering Research Council.
Sig. :	Sigmoid colon.
S.R.S. :	Synchrotron Radiation Source.
S.T.E.M. :	Scanning, transmission electron microscopy.
T.E.M.:	Transmission electron microscopy.
Trans.:	Transverse colon.
U.V.:	Ultraviolet.
W.L.F. :	Weaned low fibre fed group.

Table of Contents

Chapter 1

The structure of the colon

and related disease.

1.1 An overview of human colonic structure.

The structure of the human bowel wall has been neglected in the study of colonic disease. Research on colonic diseases has concentrated on the epidemiology and treatment of these conditions, without adequate analysis of the origin of the abnormality. The experimental work presented in this thesis attempts to study fundamental structural abnormalities in colonic collagen which may occur in diverticular disease.

Description of the human colon.

The large intestine extends from the lower pole of the caecum to the anus, and is approximately 150 cm long. The diameter is much greater than that of the small bowel and diminishes gradually from the right colon to the rectum.

The **caecum** is that part below a horizontal line across the bowel at the level of the ileocaecal valve.

The **ascending colon** extends from the horizontal line described above, to the hepatic flexure of the transverse colon.

The **transverse colon** crosses the abdomen from the hepatic flexure to the splenic flexure.

The **descending colon** is that part of the left colon from the splenic flexure to a point where it crosses the brim of the pelvis.

The **sigmoid colon** extends from the latter point to the recto-sigmoid junction which is conventionally defined as being opposite to the promontory of the sacrum.

The position of all of these sections in relation to each other is illustrated in Figure 1.1.

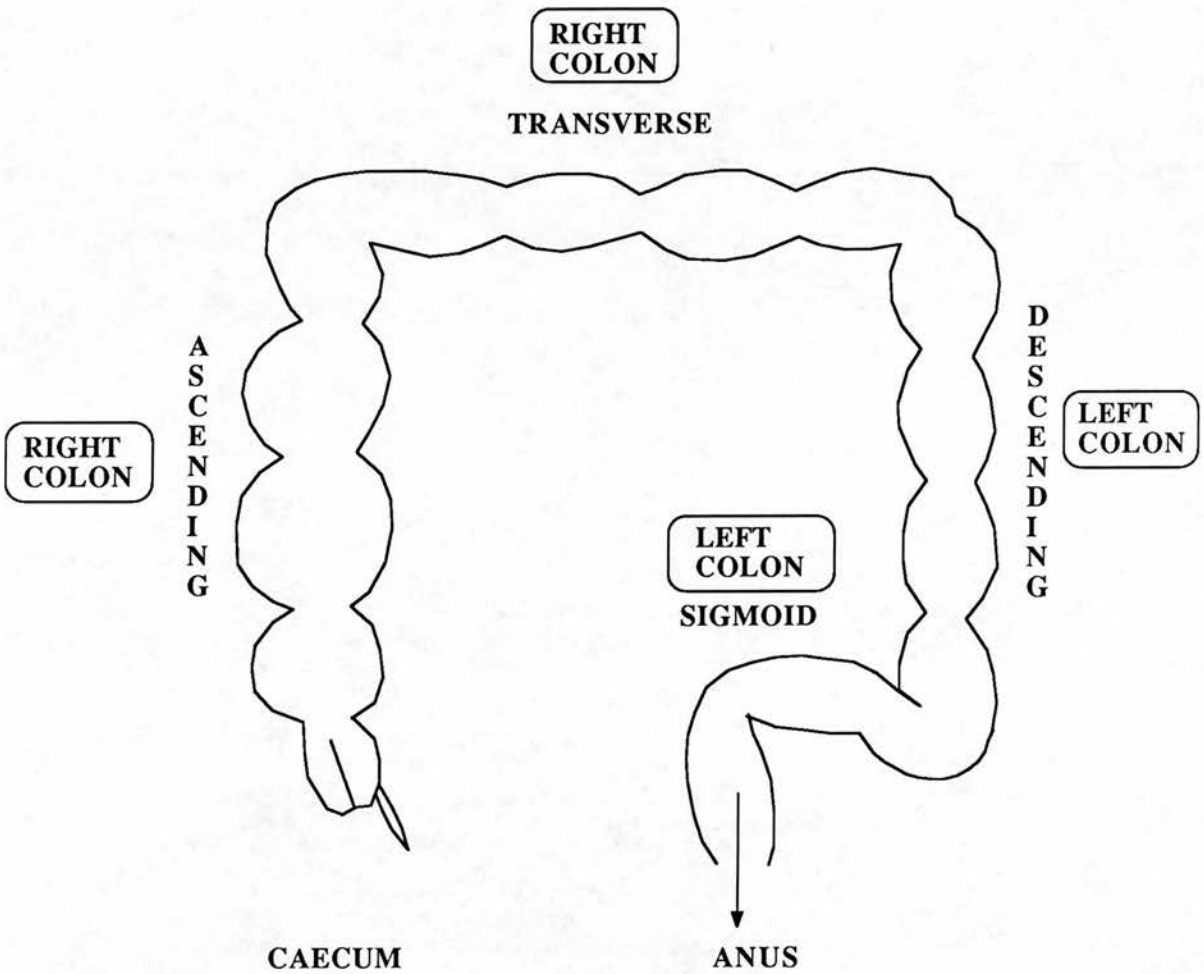


Figure 1.1 : Diagram representing the human colon from caecum to anus. This illustrates the relative positions and proportions of each of the four designated regions. The four regions are (from right to left) ascending, transverse, descending and sigmoid respectively.

The caecum is completely covered by peritoneum. The ascending colon, hepatic flexure and descending colon are covered on their anterior aspects only. The transverse colon and the sigmoid colon are completely invested, apart from their mesenteric attachments. The rectum is covered by peritoneum on the anterior and lateral aspects of the upper third, and only anteriorly in its middle third. The lower third of the rectum is entirely subperitoneal. The colon from the caecum to the rectosigmoid is covered by numerous appendages called the appendices epiploical.

1.1.1 Cross-sectional view of the human colon.

The wall of the colon and rectum includes the mucous membrane. This consists of the muscularis mucosae and muscularis propria. These regions are separated by a submucosal layer of loose connective tissue. The muscularis propria has two layers. The inner layer consists of fasciculi of circular fibres forming a continuous sheath around the whole of the large intestine. The outer, or longitudinal muscle, also forms a continuous sheath. This is very thin, except where it is concentrated into the three thick, narrow bands or teniae coli. The thick muscular bands vary in their position relative to the circumference of the colon in different parts of the large bowel. They fuse in the region of the recto-sigmoid about 10cm above the peritoneal reflection. The teniae coli have the function of shortening the colon as they are shorter than the intestine to which they belong. Consequently, the circular muscle is puckered into sacculations called haustra. The teniae coli act as strong longitudinal cables on which the circular muscle is fixed. The mucularis propria contains a considerable amount of elastic tissue, which increases with age. Animals with no teniae, such as the dog, contain very little elastic tissue.

1.2 Colonic submucosa.

The colonic submucosa is composed of connective tissue, including both collagen and elastic fibres. A plexus of veins, lymphatics and small groups of ganglion cells lie just beneath the muscularis mucosae. A scanning electron microscopy (S.E.M.) study has shown that the collagen and elastic fibres of the small intestine from rats are arranged in the form of a pliable network which has great natural strength. This layer is therefore important in the construction of intestinal anastomoses in gastrointestinal surgery (Komuro T., 1988). Gabella described a characteristic lattice pattern of collagen fibres in the small intestine of a number of species as observed by transmission electron miroscopy (T.E.M.) (Gabella G., 1987). The lattice consisted of two arrays of fibres running diagonally in a clockwise and anticlockwise direction, making an angle of $50\text{-}55^0$ with the longitudinal axis of the small intestine. The collagen lattice was found to be flexible. The angle between fibre arrays changed with the movements of the intestine. This study examined small intestinal submucosa from rats, guinea pigs, rabbits and sheep. A similar pattern was also observed in a study using T.E.M. This examined submucosal collagen from the large intestine of humans and laboratory rats (Thomson H. J. *et al*, 1987a). The lattice pattern of collagen fibrils was found to be similar in the human and the rat colon wall.

The muscularis propria is pierced at regular intervals by the main arterial supply and venous drainage of the mucous membrane. These are points of natural weakness where the submucosal layer is very thin. It is these points through which inflammation and diverticula may penetrate. It has been accepted for a century that the colonic submucosa is the layer of the colon wall which is most resistant to shear and stretching forces (Halsted W.S., 1891). Watters *et al*, used biomechanical techniques to examine the "bursting strength"

and tensile strength of the human colon wall (Watters D.A.K., 1983), (Watters D.A.K. *et al*, 1985). *Post-mortem* colon samples from Edinburgh and Kampala, Uganda were examined to determine any differences between the western civilised colons and those from the less developed countries. This experiment was designed to examine the effect of diet on the strength of the colon wall, as the difference in diet between the two countries is vast. Less developed countries eat a diet rich in dietary fibre and low in processed, refined foods. Developed western countries normally eat a diet which generally contains a high proportion of refined, processed foods which are very low in dietary fibre. These differences would give rise to different transit times and stool weights. This would influence the function of the colon wall and potentially, the structure of the colon wall. These experiments demonstrated that the tensile strength and integrity of the human colon wall are dependent on the submucosal layer. They also illustrated that the tensile strength of the human colon declined with age. A reduction in the diameter of the colon was observed with age.

A technique which allowed the isolation of the intact human colonic submucosa has been developed (Thomson H.J. *et al*, 1987a). This technique was utilised in this thesis. The same group later studied the submucosa in more detail, using T.E.M. (Thomson H.J. *et al*, 1987b). Collagen fibril abundance, and diameter, in normal colonic tissue and colonic diverticulosis affected tissue was investigated. Results indicated that the collagen fibrils of the left colon (descending and sigmoid regions) were more closely packed and smaller in diameter than those of the right colon (ascending and transverse regions) in the normal subjects. The right side of the colon had an average of 64+/-10 fibrils in a given area compared with 78+/-9 fibrils in the left side of the colon. This effect was more pronounced in the subjects with colonic diverticulosis. The

conclusion drawn from these results was that the left colon is subjected to considerable mechanical stress, more so than the right colon. Therefore a stronger, more resistant colon wall may be required in this region. The increased strength required could be produced by a tighter collagen network in the submucosa.

Colonic submucosal collagen has been neglected in research. This thesis attempts to remedy the situation by studying structural aspects of normal and diseased colons.

1.3 Colonic diverticulosis.

Diverticular disease of the colon is classified as the presentation of colonic diverticula. These are herniations of the colonic mucosa through the colonic muscle wall. Diverticular disease is an acquired condition. Although diverticular disease was initially described as an inflammatory disease (diverticulitis), it soon became obvious that there was an earlier and much more important non-inflammatory phase (diverticulosis) related to a structural weakness in the bowel wall. This weakness is determined by the point of penetration of blood vessels, the vasa recta, through the colon wall.

Burkitt and Trowell, popularised the concept that the fibre deficient diets associated with western lifestyles, contributed to the development of gastrointestinal disorders. These include hiatus hernia, appendicitis, gallstones, colonic diverticular disease, constipation and bowel cancer (Burkitt D.P. and Trowell H.C., 1975). This concept was widely accepted to explain the effect of fibre deficiency on the gastrointestinal tract. However, a report published by the British Royal College of Physicians (1980), concluded that the link between dietary fibre and gastrointestinal disorders has not been

established. The dismissal of this concept does not explain why populations with high fibre intakes show a lower incidence of the disorders listed above than those with low fibre intakes.

Morson changed the emphasis of study by noting that the predominant feature of the sigmoid colon in diverticulosis of the colon, was a thickening of the muscle layers (Morson B.C., 1963). A possible muscle dysfunction was already receiving attention, as studies had shown that there was raised colonic intraluminal pressure in diverticular disease (Painter N.S. and Truelove S.C., 1964). This led to the suggestion that the diverticula developed as an extrusion of the mucosa through the muscle coat.

The obvious muscle thickening, and the demonstration of high intraluminal pressures by some groups of workers, have inspired many to regard diverticular disease as a disorder of function. Painter discussed the possible contribution of obesity, ischaemia and the site of entry of blood vessels to structural disorders of the colon (Painter N.S., 1975). There is little evidence to support the roles of obesity and ischaemia, but the relation of diverticula to the point of entry of blood vessels in the bowel wall is well recognised (Drummond H., 1917). Two rows of diverticula, one on each side of the colon, emerging between the mesenteric and antimesenteric taeniae have been described (Slack W.W., 1966). These are illustrated in Figure 1.2.

Claims have been made that diverticular disease followed a dramatic fall in the consumption of dietary fibre in the western world in the 20th century (Painter N.S. and Burkitt D.P., 1971). Modern research has thus sought a role for fibre deficiency as an aetiologic factor, and for fibre itself as a means of modifying the physiopathologic disturbances. The decrease in dietary fibre

intake, leads to less water uptake and a less bulky dry stool. This is inherently more difficult to propel along the large bowel, and results in raised colonic intraluminal pressure. Colonic diverticulosis has become an ever increasing clinical problem, being the most common disorder of the colon in the modern western world. This condition is common in most, developed countries in, northern Europe, north America and Australia. Diverticular disease is less common in southern European countries and very rare in Africa, India and Japan.

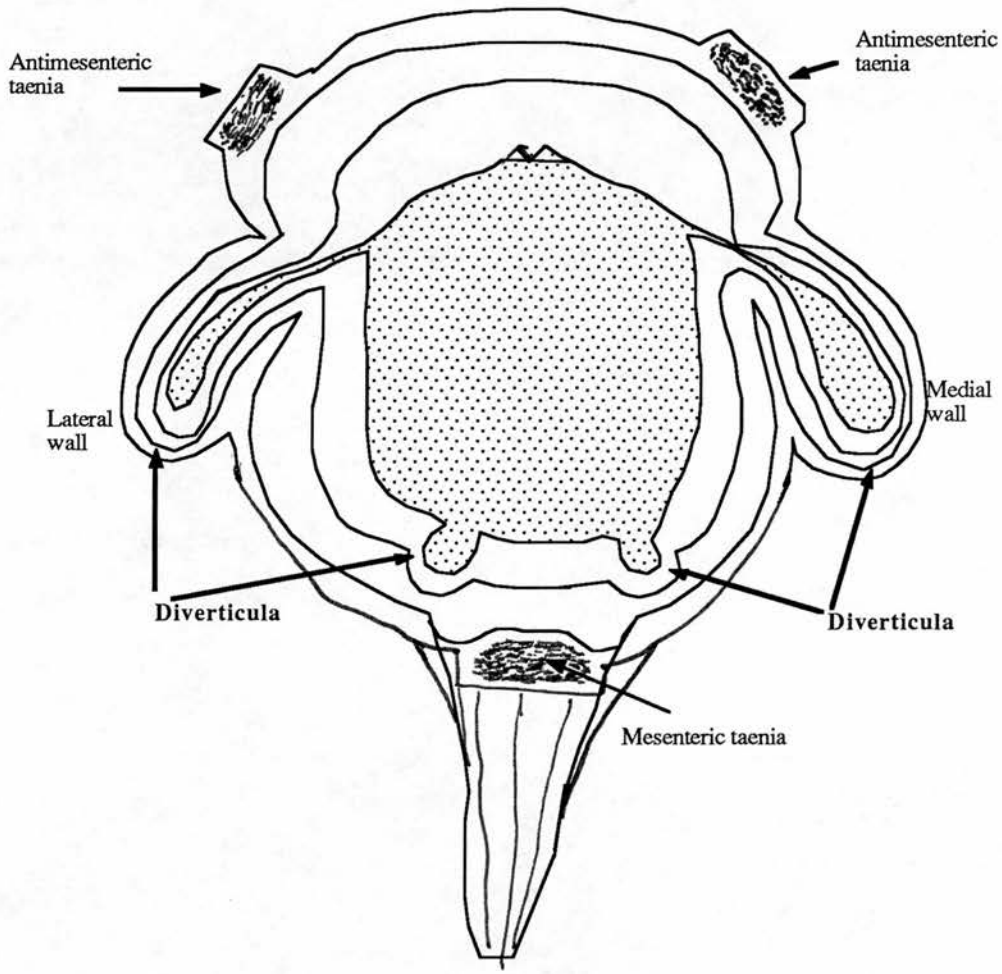


Figure 1.2 : This is a diagrammatic representation of a transverse section of the human colon wall. The predominant location of colonic diverticula, at the point of penetration of blood vessel at the mesenteric and antimesenteric taeniae are shown. Two rows of diverticula are illustrated, one on each side of the colon, this being the predominant clinical feature.

1.3.1 Terminology.

Terminology is very important in the description of this disease and the recognised terminology is outlined below.

Diverticular disease is the most satisfactory generic expression to describe the disorder when it is accompanied by characteristic muscle abnormality. This is particularly true for the sigmoid colon.

Diverticulosis indicates the presence of multiple diverticula with or without the accompanying muscle abnormality found in sigmoid diverticular disease.

Diverticulitis is the term applied when one or more diverticula are the source of inflammation and pericolic abscess formation.

Colonic diverticulosis can also be described in three different forms.

(1) **Colonic diverticulosis.** This is described as an inevitable development associated with advancing age and is not associated with symptoms. It is a benign concommitant of the aging process.

(2) **Symptomatic diverticulosis.** This is described as the presentation of diverticula associated with colonic spasm and increased colonic pressure.

(3) **Complicated diverticulosis.** This is described as the presentation of diverticula associated with colonic bleeding, perforation, fistula and pericolic abscess formation.

The incidence of each of these conditions decreases from (1) to (3) above, from 30% of the population with colonic diverticulosis to 0.01% with complicated diverticulosis. Colonic diverticulosis is common in developed western populations. It presents most frequently on the left side of the colon, especially the sigmoid colon. Diverticulosis of the right colon is uncommon except in some eastern populations (Ohi G., Minowa K. and Oyama T., 1983), (Sugihara K. *et al*, 1984).

Colonic diverticula are of a pulsion type, with a pouch of mucous membrane

projecting through the circular muscle layer to the pericolic fat and appendices epiploicae. These occur on the underside of the colon between the mesenteric and medial and lateral mesenteric taeniae. The development of colonic diverticulosis may progress in a number of ways. There may be changes in the colonic wall which are a feature of the aging process (Watters D.A.K. *et al*, 1985). Prolonged increased intraluminal pressure in the sigmoid colon may lead to pulsation diverticula developing in the rectosigmoid colon. Colonic diverticulosis implies that diverticula are present. It is unfortunate that the term diverticulitis developed, as the problem is one of spasm rather than of inflammation.

1.3.2 Theories relating to the aetiology of colonic diverticulosis.

Painter proposed that colonic diverticulosis is due to a deficiency of dietary fibre. The colonic muscle requires to contract strongly in order to transmit and expel the small stool associated with a fibre deficient diet (Painter N.S., 1975). The increased pressure within the segmented section of the bowel may result in herniation. There are undoubtedly differences in prevalence of diverticular disease between England, Wales and Scotland. There are also differences between different regions of Scotland, to an extent parallel to differences in fruit and vegetable intake (Eastwood M.A., Sanderson J. and Pocock S.J., 1977).

Painter developed a theory to explain the predominance of diverticula in the sigmoid colon (Painter N.S., 1975). He stated that faeces move round to the sigmoid region, where they are held up at the narrowest part of the colon. At this site most of the faeces are halted except immediately prior to defecation. The sigmoid colon becomes segmented as it halts and returns faeces approaching from the proximal colon. In certain circumstances the colon has

to cope with an abnormal stream of faeces of increased viscosity. This may be due to a fibre deficient diet that leads to small, hard stools and would eventually hypertrophy over a number of years. This would result in herniations, (or diverticula) due to excess segmentation and pressure production.

This situation would be amplified as hypertrophy of the sigmoid muscle and development of diverticulosis would lead to narrowing of the lumen and more efficient segmentation. Increased narrowing would then lead to the development of further diverticula. In this situation the descending colon would be forced to work harder, in order to deliver the faeces to the sigmoid colon prior to defecation. This region may therefore become affected by diverticulosis. This effect would spread the condition proximally around the colon.

Painter has suggested that should this theory be correct, diverticulosis is the result of a normal colon being altered by an abnormal diet and is not due to some congenital colonic abnormality (Painter N.S., 1975). This conclusion is in accord with the clinical pattern of the disease.

Colonic diverticulosis was the first disease in which a high fibre intake was claimed to have a therapeutic role (Painter N.S., Almeida A.Z. and Colebourne K.W., 1972). Fibre treatment seemed logical, as the leading theory for the pathogenesis of diverticulosis was that the colon developed an herniation as a result of propelling abnormally small, hard stools (Painter N.S. *et al,* 1965), (Painter N.S. and Burkitt D.P., 1971). This theory has been tested in several ways and has survived, albeit with some anomalies. A dietary fibre theory, is supported by the evidence that colonic diverticulosis is rare in black Africans

but common in black Americans (Burkitt D.P., Clements J.L. and Eaton S.B., 1985). Few black Africans live long enough to develop what is essentially a condition of the elderly.

Accurate data are not available for sufficient populations to enable comparisons to be made between prevalence of colonic diverticulosis and dietary fibre intakes. However in England the condition is substantially less common in vegetarians (Gear J.S.S., Ware A. and Fursden P., 1979). Vegetarians generally eat twice as much dietary fibre as the non vegetarian population; they also pass substantially bulkier and softer stools. In Japan, dietary fibre intake has fallen steadily since World War II. Autopsy surveys suggest that the prevalence of colonic diverticulosis has risen. Case-controlled studies in Japan show people with colonic diverticulosis to have been in the habit of eating less fibre than matched healthy controls (Nagahashi M. *et al*, 1985). This trend is also apparent in Greece, where Manousos reported a significant reduction in the relative risk of colonic diverticulosis. He found that this was associated with doubled consumption of four fibre rich food items: brown bread, spinach, lettuce and cucumber (Manousos O.N., Day N.E. and Tzonou A., 1985).

These case-control studies appear to be convincing, but are hard to interpret as the cases of diverticulosis were all symptomatic hospital patients. Since only a minority of people with colonic diverticulosis have symptoms and fewer still are referred to hospital, the results may be biased and cannot safely be extrapolated to the generality of cases. There is only one case-control study based on asymptomatic cases, detected by population screening and the results were not positive (Gear J.S.S., Ware A. and Fursden P., 1979). Fibre intake was lower in cases only if the subjects were over the age of 60 and the authors had to resort to the suggestion that the younger controls were contaminated by

people in a prediverticular state. This state can be described as the presentation of small diverticula lying in the mucosa of the colon wall, without protruding into the serosa. Research by Eastwood *et al*, revealed that 24 hour stool weight was no lower in patients with colonic diverticulosis than in the general population (Eastwood M.A. *et al*, 1978b).

If starch contributes significantly to faecal bulk, one might expect an inverse relationship between starch intake and the risk of colonic diverticulosis. This has not been reported to date. Thornton *et al,* found that patients with colonic diverticulosis were unusually efficient in absorbing the starch in a potato test meal. This implies that they allow less starch into the colon (Thornton J.R. *et al*, 1986). This area requires further study.

Bran, a high fibre diet or bulking agents are still considered to be the most successful treatment for symptomatic diverticulosis by most gastroenterologists. The results from controlled placebo trials are not unanimous, this may be due to the cases studied. Bran, as a treatment for colonic diverticulosis was markedly superior to placebo in a trial of surgical patients (Brodribb A.J.M., 1977). Bran was no better in a trial of medical patients (Ornstein M.H. *et al,* 1981). The latter trial was criticised for having too low a dose of dietary fibre (Heaton K.W., 1985). It has been postulated that the real problem may be that symptomatic diverticulosis is irritable bowel syndrome in a person who has diverticulosis (Thompson W.G. *et al*, 1982).
Colonic diverticulosis is a benign condition associated with an elderly population and should be regarded as a frequent feature of the aging process. Colonic diverticulosis is rare before the third decade in the United Kingdom, however a third of the population have colonic diverticulosis by the 6th decade and a half by the 9th decade (Painter N.S., 1975). It is therefore possible that

the increased incidence of colonic diverticulosis with age is secondary to a decline in the mechanical integrity of the colonic wall. Whiteway and Morson, noted a thickening of the circular and longitudinal muscle coats. This was associated with a progressive increase in the thickness of collagen, elastin and reticular tissue of the colon wall (Whiteway J. and Morson B.C., 1985a), (Whiteway J. and Morson B.C., 1985b).

Mechanical properties of the colon depend on the strength, flexibility and integrity of the individual elements and on their relative orientation and interaction. These viscoelastic properties give the colon expansibility, strength, low dissipation and maintenance of shape. It has also been shown by T.E.M. that the collagen fibrils from the colons of those subjects with colonic diverticulosis are similar to those of normal individuals of an older age (Thomson H.J. *et al*, 1987b). This suggests that colonic diverticulosis may be due to an enhanced aging process in the colonic submucosa. Painter and Arfwidsson have individually shown that the segmenting of the sigmoid colon was greater in patients with diverticulosis than in control subjects (Painter N.S., 1975), (Arfwiddson S., 1964). These segmenting contractions allow mixing of the colonic contents. Increased contractions are thought to be the source of pain and underly the feeling of obstruction which is a complaint of symptomatic subjects. In symptom free subjects, the segmenting pressures are normal (Eastwood M.A. *et al*, 1978a).

No relationship between increased haustral patterns in the colon outlined by barium enema examination, and high pressure activity in the colon has been found (Weinreich J. and Andersen D., 1976). Patients with colicky sigmoid syndrome and chronic colonic diverticulosis were studied. The first condition was characterised by lower abdominal pain, but no colonic diverticula, and

included the irritable bowel syndrome. There was no relationship between the presence of diverticula and high pressure activity. This is suggestive that colonic diverticulosis is complicated by high pressure, rather than that high pressure is a causative element in the aetiology of the condition.

When wheat bran is given to individuals with increased pressure in the colon, the result is an increase in stool weight and a decrease in intracolonic pressure (Findlay J.M., Smith A.N. and Mitchell W.D., 1974). In these subjects 20g of unprocessed wheat bran shortened the intestinal transit time in colonic diverticulosis whilst increasing stool weight.

It is of interest that wheat bran increases stool weight by an average of 2.5g wet weight faeces per gram fibre, whereas in subjects with colonic diverticulosis the increase is of the order of 0.9g wet weight faeces per gram fibre. With unprocessed wheat bran (16-20g) the stool weight increased by 60.0g per day in normal controls, and by 17.5g per day in patients with colonic diverticulosis. The effect on the motility index in the diverticulosis patients was to decrease basal pressure. Wheat bran, however, increased stool weight by a modest amount and reduced transit time and colonic motility.

Isphagula (traded in Britain as a medication for the treatment of colonic motility disorders, as Fybogel) appeared to increase stool weight, but also increased the basal motility index. This had no effect on the food stimulated pressures (Eastwood M.A. *et al*, 1978a). When isphagula was fed at a dose of 7g per day was found to increase stool weight by a mean of 28g per day. The increase in wet stool weight is 4-6g wet stool weight per gram isphagula.

The physical nature of the administered wheat bran is also important, coarse bran being more effective than fine bran (Kirwan W.O. *et al*, 1974), (Brodribb A.J.M., 1981). The effectiveness of wheat bran in the treatment of symptomatic diverticulosis is not affected by the origin of the bran. One study examined the effectiveness of two types of wheat, Canadian Red Spring Wheat which is used for bread making and French Soft Wheat which is commonly used for cakes (Smith A.N., Drummond E. and Eastwood M.A., 1981). Cooking may also decrease the potency of a wheat bran fibre on the colon in humans (Wyman J.B. *et al*, 1976).

1.4 Appearance of tissue affected by colonic diverticulosis.

Muscle abnormality is the most consistent and striking abnormality in colonic diverticulosis (Morson B.C., 1963), (Hughes L.E., 1969). The teniae coli appear thick, assuming an almost cartilaginous consistency. The circular muscle is also much thicker than normal, with a concertina (or saw tooth) like appearance. The concertina, or saw tooth sign observed on barium enema radiographs, is the consequence of shortening of the bowel. The thickened circular muscle layer is thrown into alternating semilunar arcs of muscle confined to the zone between the mesenteric and antimesenteric teniae. In between the muscular corrugations, the mouths of diverticula are found penetrating the bowel wall to reach the pericolic fat.

With the advent of cineradiography, coupled with the means of measuring the intracolonic pressures accurately, it became possible to show that diverticula were associated with high localised intraluminal pressures (Edwards H.C., 1939). The living colon can segment to such an extent that it obstructs its lumen intermittently. It then functions not as a tube but as a series of small bladders (Painter N.S. *et al*, 1965).

Diverticula are most common in the sigmoid part of the colon. More proximal involvement only occurs when the sigmoid colon is also affected. Diverticulosis of the total colon is uncommon and usually symptomless. The rectum is never involved. A diagram illustrating the incidence of diverticula along the length of the colon is shown in Figure 1.3.

1.5 Epidemiology of colonic diverticulosis.

Diverticular disease is rare in Oriental countries. A table of the relative incidence of this condition is outlined in Table 1.1. The vast majority of those affected have no symptoms despite the presence of advanced radiological changes in the sigmoid colon. Many groups have concentrated on examining the epidemiology of colonic diverticulosis (Mendeloff A.I., 1986), (Mendeloff A.I., 1987), (Manousos O.N., 1989).

It is common to find two rows of diverticula, one on each side of the colon. However, in approximately 50% of all cases a third row of diverticula can be found between the two antimesenteric taeniae (Watt J. and Marcus R., 1964).

1.5.1 Rising incidence of colonic diverticulosis following westernisation of the diet.

The incidence of diverticulosis has risen dramatically in the United Kingdom, the United States, Australasia and western Europe in the last forty years. Diverticular disease was almost unknown in 1900 but is now the most common disorder of the colon in western man. The diagnosis of colonic diverticulosis depends upon radiology. The increased usage of barium enemas may have increased the number of diagnoses. This change in incidence has happened in only 90 years which is close to the average lifespan of man.

Diverticulosis is almost unknown to this day in communities who have not adopted western lifestyles and who have adhered to their traditional eating habits. Only 50 years ago, diverticulosis was much less prevalent in black than in white Americans, (Kocour E.J., 1937) however this difference has almost disappeared (Cleave T.L., Campbell G.D. and Painter N.S., 1969). Similarly the disease is seen in Asians, Africans and West Indians who have lived in Britain for many years. The disease was rare in Japan until recently, however the prevalence of colonic diverticulosis is increasing due to changes in dietary fibre intake (Ohi G., Minowa K. and Oyama T., 1983). One striking piece of evidence, is that diverticulosis is as prevalent in American Japanese who were born and bred in Hawaii as it is in white Americans (Stemmerman G.N., 1970). The few cases reported in India and Iran have been found mostly in the wealthy patients. In Johannesburg, diverticular disease has been found in black patients only in the last 15 years. All of those affected were educated and had adopted a western lifestyle (Segal I., Soloman A. and Hunt J.A., 1977). These facts indicate that the disease is not racial but environmental in origin.

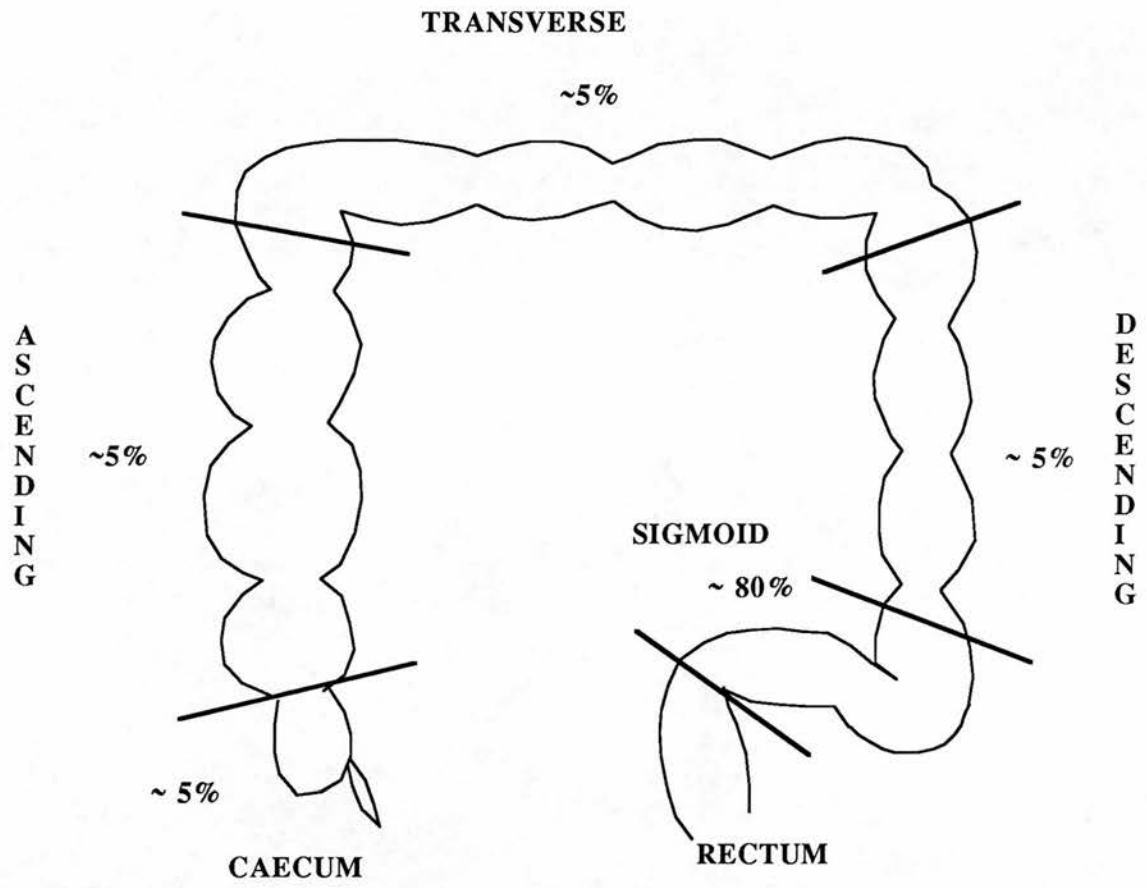


Figure 1.3 : This illustrates the four regions of the human colon and the relative value for the incidence of colonic diverticulosis in that region. The values are shown as percentages of the total incidence. It is apparent that the sigmoid region is the predominant location for the development of colonic diverticula.

Country and origin	Number Affected	% Affected	Total Examined	Comments on age
United Kingdom				
Edwards (1953)	25	1.6	1623	All over 35
Manousos (1967)	38	35	109	All over 60
Sweden	87	4.2	2090	
France	200	40	500	40% over 70
United States	1398	5.7	24620	
	600	30	2000	
Africa				
Painter N (1975)	1	0.05	2000	Higher frequency in urban communities

Table 1.1 : This table illustrates the relative incidence of colonic diverticulosis from various countries and continents of the world. Both western, developed countries and less developed countries are shown. It is apparent that the incidence is markedly lower in the less developed countries.

1.6 Dietary influences.

The dramatic increase in the incidence of diverticular disease in the western world can theoretically be explained in two ways:

Proposal 1: The increase may be due to observer error and be more apparent than real. This is unlikely because the quality of writing shows that the clinicians and pathologists of the last century would have recognised diverticulosis had it been common in their day (Morson B.C., 1963).

Proposal 2: The increase may be due to the fact that the environment of the colon has changed and that diverticula are caused by our modern diet. In particular this applies to the low intake of dietary fibre, since this is the fraction of food which reaches the colon least altered by digestion. The amount of fibre in the diet has a direct effect on the intestinal transit time, the weight and consistency of the stools (Burkitt D.P., Walker A.R.P. and Painter N.S., 1972), (Painter N.S., 1975). Another effect of dietary fibre on the colon is alteration of the pressures within the colon (Srivistava G.S., Smith A.N. and Painter N.S., 1976), (Smith A.N., Shepherd J. and Eastwood M.A., 1981).

1.6.1 Dietary fibre intake.

It is necessary to define dietary fibre, as the exact definition has become a controversial subject over recent years. Dietary fibre had previously been called roughage and was considered a gastrointestinal irritant. This is now known to be incorrect. Dietary fibre has also been called unavailable carbohydrate by British food analysts.

All constituents of the diet can be named and defined in terms of their chemical composition. The group of substances which come under the general

classification of dietary fibre, pose different problems to food scientists, biochemists, botanists, cereal chemists, nutritionists and gastroenterologists alike. At a fundamental level, dietary fibre has been chemically defined as, "the sum of the polysaccharides and lignin which are not digested by the endogenous secretions of the human gastrointestinal tract; this fraction has a variable composition and is made up of different types of polysaccharides (cellulose, hemicelluloses and pectic substances) and lignin". This definition was tendered by Paul and Southgate and is now the recognised definition. There is still some controversy over the inclusion of resistant starch in this category (Paul A.A. and Southgate D.A.T., 1978).

Cereal fibre intakes have been falling in Britain during the last 200 years. Wheat flour intakes have declined from an estimated intake of over 400 pounds per head per year in 1750, to under 150 pounds per head per year in 1950 (Hollingsworth D.F. and Greaves J.P., 1967). There are no data concerning the dietary fibre content of wheat flour in past centuries. It is certain that modern, low fibre, white flour slowly replaced the traditional high fibre, coarsely ground wholemeal flour. At the same time, the consumption of fibre free sugar and fat have risen during the last 200 years. These have replaced, in terms of energy, the declining intake of both flour and potatoes.

In England, Brodribb and Humphreys compared the crude fibre intake of 40 patients with symptomatic colonic diverticulosis, with matched controls. The mean intake was 2.6g crude fibre per day in patients with diverticulosis, and 5.2g per day in control patients. The method of measuring the dietary fibre intake was to ask the patients to recall their eating habits (Brodribb A.J.M. and Humphreys D.M., 1976). The main difference in fibre intake between the control subjects and those with colonic diverticulosis was found to be in the

cereal fibre fraction (Gear J.S.S., Ware A. and Fursden P., 1979).

Crude fibre intake has a very marked effect on the condition of the bowel. This is only apparent after a certain period of time i.e. after the age of 60, when the first symptoms of diverticular disease become apparent. The effect of dietary fibre on the health of the population is an important area of study and has been substantially reviewed (Anderson J.W., 1986).

1.6.2 Measurement of dietary fibre.

One of the major problems encountered during studies of dietary fibre intake is the accurate measurement of the dietary fibre content of a variety of foods. The dietary fibre content of most of the worlds foods is, as yet, unknown. Another problem is the diversity of techniques used to measure both dietary fibre intakes and the dietary fibre content of foods. The different techniques give rise to widely different results for the same foods. A review of the techniques involved in dietary fibre measurement was undertaken (Englyst H.N. and Cummings J.H., 1985). This review illustrated that the results obtained by different groups of researchers, using different or similar measurement techniques, showed a large variance. This suggests that results from these studies may be worthless. The review article illustrated that complex studies of human dietary habits are very problematic. The process of collecting accurate dietary records, measuring dietary fibre content of the food in the diet, and assessment of dietary fibre intake is associated with errors at almost every step of analysis (Englyst H.N. and Cummings J.H., 1985). This may consequently mean that apparent differences in dietary fibre consumption between groups, may indeed be an artifact produced by different techniques.

Another problem in human dietary studies is the assessment of other contributory factors which may influence the development of a disease. These are the influences of age, sex, environment, lifestyle, wealth and genetic predisposition, which may affect the outcome of the results. It is almost impossible to conduct a human study which is free from these external influences, and therefore the laboratory animal is used as a substitute model.

Most of the dietary fibre studies are conducted on the laboratory animal and in particular the laboratory rat. To study the influence of diet on colonic diverticulosis, a search was initiated to find a laboratory animal which would develop diverticula if fed a low fibre or fibre free diet. It was noted that laboratory rats fed a high fibre diet did not develop diverticula, while those fed a low fibre diet developed diverticula of the colon (Carlson A.J. and Hoelzel F., 1949). The laboratory rat was found to be a suitable animal model for the study of colonic diverticulosis.

As a result of this discovery, various studies have been conducted using the rat as a model to examine colonic diseases. The most significant of these was an examination of cereal dietary fibre consumption and its relation to the development of diverticular disease by Fisher *et al*,. Nine groups of rats were reared on various isocaloric diets. The variant being the concentration or source of dietary fibre. All of the rats were maintained on a lifetime study. After death the rats were dissected and examined for the presence or absence of colonic diverticula. The results showed that 48% of the rats on a low fibre diet developed diverticulosis of the colon compared to 9% of the control high fibre group (Fisher N. *et al*, 1985). This experiment produced a means of studying the effect of diet on the colon and the development of colonic diverticulosis.

1.7 The physiological effects of dietary fibre on the gastrointestinal tract.

It is known that dietary fibre has a pronounced effect on both the function and gross morphology of the gastrointestinal tract (Cassidy M.M., Fitzpatrick L.R. and Vahouny G.V., 1981). The effect of dietary fibre on the colon has been studied in detail. All of the studies have been carried out on the laboratory rat. Dietary fibre is known to affect the morphology of the colon (Jacobs L.R. and Schneeman B.O., 1981). The effect was illustrated by feeding a diet containing 20% wheat bran to rats for two weeks. The feeding of this diet resulted in the development of hyperplasia of the colonic mucosa and hypertrophy of the colonic muscle. It is now known that the proliferative activity of the intestinal mucosa is influenced in both humans and laboratory rats who have been fed this diet.

Dietary fibre also influences the production of gases in the caecum and colon due to bacterial fermentation of the fibre. The three main gases produced are carbon dioxide, hydrogen and methane. A number of studies have been carried out to examine the effect of different fibres on the gas levels and concentrations in the colon (Bond J.H. and Levitt M.D., 1976), (Eastwood M.A. and Kay R.M., 1979). Perhaps one of the most important effects of fibre on the gastrointestinal tract is the effect on the intestinal transit time. Several groups have illustrated a positive reciprocal relationship between fibre intake and transit time. Wheat bran was the most effective fibre in reducing transit time (Burkitt D.P., Walker A.R.P. and Painter N.S., 1972), (Findlay J.M., Smith A.N. and Mitchell W.D., 1974), (Spiller G.A., Chernoff M.C. and Shipley E.A., 1977).

The physical nature of the fibre is also important. Raw bran which has a high

water holding capacity is more effective in reducing transit time than fine bran or cooked bran (Eastwood M.A. *et al*, 1978a), (Smith A.N., Drummond E. and Eastwood M.A., 1981). In conjunction with the reduced transit time, dietary fibre increases the faecal output. The relationship between dietary fibre and stool weight has been substantially reviewed in the past (Eastwood M.A., Watters D. and Smith A.N., 1982). The exact value for normal stool weight is unclear and depends very much on the individual involved, their diet, environment and lifestyle. Estimates of stool weight for the average western diet are 100-150g/day (Eastwood M.A., Watters D. and Smith A.N., 1982). Vegetarians have a higher faecal output, at approximately 225g/day (Burkitt D.P. and Trowell H.C., 1975). Fibre exerts a great number of influences on the colon and the study of these influences is paramount. Table 1.2 illustrates the diversity of the sources of dietary fibre and the vast differences in the proportion of dietary fibre found in foodstuffs.

Cummings *et al*, have pointed out that a doubling of faecal weight is required to induce substantial decreases in intraluminal pressure in the distal colon (Cummings J.H. *et al*, 1978). It is increased pressures which may potentially lead to a damaged colon wall. It is important to inform the population that dietary fibre is essential to prevent diverticulosis. The recommended daily intake is 20-25g of dietary fibre.

Diverticulosis is becoming an ever increasing clinical problem in western societies. Research into this disorder is required to determine the mechanism by which it occurs. This may aid the treatment and prophylaxis. This thesis aims to study the structural aspects of collagen on the development of colonic diverticulosis in the colons of both humans and the laboratory rat. The effect of age and diet on colonic collagen and colonic diverticulosis will be examined.

Source	Grams required to produce 20g of dietary fibre and double the daily stool weight
Unprocessed bran	45g
Wholemeal bread	404g
Whole carrots	681g
Cabbage	775g
Apples (fresh)	1477g

Table 1.2 : This table illustrates the amount of a foodstuff required to produce 20g of dietary fibre. This is the amount which is considered to be the daily requirement to double the stool weight. Bran in its most natural form appears to be the most efficient substance for stool bulking. The efficiency drops drastically with fresh fruit and vegetables.

Chapter 2

Introduction to connective tissue.

2.1 Review of connective tissue.

Connective tissues are essential for the spatial organisation and maintenance of structure in most forms of multicellular life. Different forms of connective tissue exist. Cellulose is the major extra cellular component in plant life, whereas chitin plays this role in insects. Collagen is the main component of connective tissue (extracellular matrix) in animals. This is the most abundant protein in mammals. Collagen occupies a key position in the molecular architecture of higher animals. It ensures the structural integrity of the organism by forming a strong scaffold to which cells or organs are attached.

The overall importance of collagen has led to a vast number of studies. These have examined, structural aspects of collagen, its ability to crosslink, connective tissue diseases and the organisation of the protein in the body. Two general points were found to be fundamental to the understanding of the role of collagen in the extracellular matrix.

(1) It has been established that a number of genetically distinct collagens exist. The term collagen applies to a family of proteins with similar characteristics.

(2) The relationship between collagen and other components of the extracellular matrix varies. This gives rise to different morphology of the tissues.

2.2 Definition of a collagen.

There are a number of criteria that define a molecule as a member of the collagen family. The most important of these is the amino acid sequence. Collagen is characterised by a repeating tripeptide, -GLY-X-Y-. The presence of a glycyl residue as every third amino acid is essential for triple helix

formation. Residues X and Y are variable amino acids, but often found as proline or hydroxyproline. The consequence of this repeating sequence in physical terms is the formation of the characteristic rope like structure of the collagen triple helix. To date fourteen collagen types have been identified and characterised in sufficient detail to warrant their classification in the collagen family (Vuorio E. and de Crombrugghe B., 1990), (van der Rest M. and Garrone R., 1991). The different collagen types are designated Roman numerals I-XIV. In all of these molecules the major component of the protein is a triple helical structure of three polypeptide chains (α chains). Other specific features of members of the collagen family include a high content of proline, alanine and lysine residues. Extensive post-translational modifications of collagen include, hydroxylation of proline and lysine residues, and enzymatic glycosylation of hydroxylysine residues. The collagen molecule is also able to form inter- and intra- molecular crosslinks through lysine and hydroxylsine residues.

2.2.1 Genetically distinct collagens.

The first conclusive evidence that different types of collagen exist within a given organism was produced in 1969 (Miller E.J. and Matukas V.J., 1969). They used procedures to solubilise collagen from chicks and found a collagen which could be characterised differently from those found previously in skin and bone. This marked the advent of the discovery of a number of distinct collagens. These collagens, although containing the central characteristics attributable to the collagens, are genetically, chemically and immunologically different from each other. The composition and the primary locations are shown for each of the fourteen classified collagens on Table 2.1.

Type	Chain	Major tissue distribution
I	$[\alpha1(I)]_2, \alpha2(I)$	Skin, tendon, bone and cornea.
II	$[\alpha1(II)]_3$	Cartilage, vitreous humour, intervertebral disc.
III	$[\alpha1(III)]_3$	Skin, lung, arteries, uterus.
IV	$[\alpha1(IV)]_2,\alpha2(IV)$	Basement membrane.
V	$\alpha1(V),\alpha2(V),\alpha3(V)$	Placenta, skin, bone and cornea.
VI	$\alpha1(VI),\alpha2(V), \alpha3(VI)$	Blood vessels, uterus, ligament. Skin, lung, cornea and tendon.
VII	$[\alpha1(VII)]_3$	Skin,and also between epithelial membranes and stroma.
VIII	$\alpha1(VIII)$ [composition unknown]	Descements membrane and endothelial cells.
IX	$\alpha1(IX),\alpha2(IX),\alpha3(IX)$	Cartilage, vitreous humour and intervertebral disc.
X	$[\alpha1(X)]_3$	Cartilage growth plate.
XI	$\alpha1(XI),\alpha2(XI), \alpha3(XI)$	Cartilage.
XII	$[\alpha1 (XII)]_3$	Embryonic tendon and skin.
XIII	$\alpha1 (XIII)$ [composition unknown]	Endothelial cells.
XIV	?	Foetal skin and tendon.

Table 2.1 : This table illustrates the family of collagen types. All of the currently classified collagens are shown together with the α chain composition and the predominant location in mammalian tissues (Vuorio E and de Crombrugghe B., 1990), (van der Rest M. and Garrone R., 1991).

Based on their supramolecular structures, the collagens are divided into two main classes: fibril forming or non-fibril forming collagens. The fibril forming collagen class contain molecules with long continuous triple helices, which are the constituents of banded collagen fibrils. The non-fibril forming collagen class are more heterogeneous. These have been further classified according to their molecular characteristics, supramolecular structures, and the types of extracellular networks that they form. From protein and gene structure information, collagen types I, II, III, V and XI have been assigned to the fibril forming group. These collagen types form highly organised fibres and fibrils. This provides structural support for the body in the skeleton, skin, blood vessels, cartilage, nerves, intestines, and in the fibrous capsules of organs.

Type I collagen, is the most abundant member of the collagen family and is the major component of tendon. It can also be found in large amounts in skin, bone, cornea, annulus fibrosis and placental tissue. This was the first collagen to be identified and is often found associated with other type I molecules in a supramolecular organisation, as fibrils. These exhibit a characteristic banding pattern of high and low electron density with periodicity 64nm. This pattern can be seen by electron microscopy.

Type II collagen is the major structural protein of cartilage. It is also known to exhibit a characteristic supramolecular banding pattern on examination by electron microscopy. This has been most successfully observed in the notochord sheath of the lamprey, where a highly aligned fibril structure exists (Eikenberry E.F. *et al*, 1984). Type II collagen has also been observed in annulus fibrosus, nucleus pulposus and the vitreous body of the eye.

Type III collagen occurs in various tissues, notably skin, bone and blood

vessels. Type III collagen contains disulphide bridges. This collagen produces a fibrous banding pattern on examination by electron microscopy. It is generally less well ordered than those structures composed of type I collagen. The demonstration of covalent lysine-derived crosslinks between type I and type III collagen molecules, was the first evidence that the two molecules may actually be part of the same aggregate (Henkel W. and Glanville R.W., 1982). *In vivo* immunochemical localisation studies have shown that types I and III are present as heterotypic fibrils (Keene D.R. *et al,* 1987). Types I and III collagen are most relevant to the work in this thesis as they have been detected in the wall of the small intestine (Epstein E.H. and Munderloh N.H., 1975).

The nature of types IV-XIV collagen, their location, and contribution to structural integrity, is less well defined, see Table 2.1. The vast literature on all of the different collagens has been reviewed (Miller E.J. and Gay S., 1987), (Burgeson R.E., 1988). Type IV collagen is known to have an essential role in the structure and function of basement membrane. It is thought to form a network type structure. The importance of these less well defined collagens is however beginning to be recognised.

Interactions between fibrous collagen forms and the non-fibrous forms are a more recent subject of study. Bovine articular cartilage contains types II, IX and XI. Every molecule of type IX collagen is covalently linked in two or more places to type II collagen. Type XI collagen is thought to cover the type II fibrils in cartilage. Other collagen types are found in cartilage. Type VI is found in the matrix in a non-fibrillar organisation, and type X is found restricted to the matrix around hypertrophic chondrocytes of the growth plate (Eyre D.R., Wu J. and Woods P.E., 1991). Collagen types I and V have

been found co-distributed within the same fibril obtained from chicken embryo corneal stroma (Birk D.E. *et al*, 1988). Heterotypic assembly of collagen has been proposed as a mechanism of regulation of collagen fibril structure and size (Adachi E., Hayashi T., and Hashimoto P.H., 1989).

2.2.2 The extracellular matrix.

The extracellular matrix does not only consist of collagens. The requirement for structural integrity demands the ability of various tissues in the body to exhibit elasticity and withstand compression. In conjunction with the collagens, there can also be found proteoglycans, fibronectin, laminin, elastin and water. It is the proportion and arrangement of these macromolecules that leads to differing biomechanical and physiological roles of specialised tissues.

Axially aligned collagen fibrils and the intrinsic rope like nature of individual molecules, confer the ability to transmit force. The tissues in which they are found can be regarded as tissues built to withstand tension along their axis, such as tendon. Tendon is a relatively simple extracellular matrix tissue. It contains a high percentage of type I collagen and relatively little proteoglycan. This can be contrasted with cartilage.

Cartilage is the smooth, wear resistant, load bearing material that forms the articulating surface of joints. It can withstand large stresses for long periods of time. The collagen network provides cartilage with its intrinsic stiffness and strength. Proteoglycan surrounding the collagen fibrils contributes static and dynamic shear properties. It therefore has a role in absorbing mechanical forces as opposed to transmitting them.

Tissues such as the wall of the colon and the aorta, contain collagen, elastin and proteoglycan. These act to produce a structure that is able to be extended without rupture under normal stress bearing loads. This allows the tissues to accommodate the strong intraluminal pressures and the pulsatile flow of blood respectively. The role of elastin here, is to allow distention of tissues without plasticity. The arrangement of collagen fibrils may also accommodate some elastic properties. This structure-function relationship is very important, as the tissues are under constant pressure and require to be able to withstand them.

The composition of the tissue is not the only factor that confers biomechanical properties. In many cases the diameter of collagen fibrils varies between tissues, and a range of fibre sizes can be characteristic of certain tissue types. The diameter of collagen fibrils has been examined in a variety of tissues. One such study illustrated that tendon exhibits a range of fibril diameters (Parry D.A.D., Barnes G.R. and Craig A.S., 1978a and b). The fibril diameter in corneal stroma is uniform and accounts for the precise packing order, and thus the high transparency, associated with the tissue (Maurice D.M., 1957), (Craig A.S. and Parry D.A.D., 1981). This particular example emphasises how the organisation of the constituent components can produce such differing structures as cornea and tendon.

The diameter of the collagen fibrils in the human colon wall has been investigated. It was discovered that the fibril diameters are different from one region of the colon to the other. This may indicate that there is a relationship between the structure and function of the collagen even within one tissue (Thomson H.J. *et al,* 1987a). Thomson's study examined full thickness specimens of the four designated regions of the human colon, (ascending, transverse, descending and sigmoid), from *post-mortem* subjects with an age

range of 9-96 years (mean 57 years). A technique was designed for the extraction of the submucosa from the other bowel coats, using a combination of sharp and blunt dissection.

The resultant electron micrographs were analysed for the diameter of the fibrils in cross section and the number of fibrils within a given area. They observed that aging of the colon was associated with smaller, more tightly packed fibrils. There were differences between the right and left colon. The submucosa from the left colon has more, tightly packed fibrils with smaller diameters. They contributed these differences to the fact that the left side of the colon is subjected to considerable mechanical stresses. The right side of the colon is under less strain. It is the left side of the colon that the high pressure motility disturbances of colonic diverticulosis have been noted (Weinreich J. and Andersen D., 1976). It was observed that there was an accentuation of the differences in collagen structure between the right and left colon in those subjects with colonic diverticulosis. This was attributed to the theory that colonic diverticulosis represents premature aging of the colon (Thomson H.J., *et al*, 1987a).

2.3 Collagen structure.

This thesis primarily describes an investigation of the structure of collagen within one type of connective tissue, the colon wall. The exact types of collagen present in the colon wall are as yet undescribed. It is almost certain, from electron microscopy evidence that the major types of collagen in the colon are members of the fibrous forming group. The colon wall has to withstand a high degree of intraluminal pressure. For this reason it would require a network of strong fibres in a base of proteoglycans and elastin to allow a high degree of strength and viscoelasticity. This survey of collagen will concentrate

on the fibrous forms of collagen. In particular type I collagen from tendon, as this is the tissue which has been most comprehensively studied.

2.3.1 Primary structure of type I collagen.

The determination of the type I collagen amino acid sequence proved to be a very difficult task. Before a full structure was elucidated, a number of features of the chemical composition were revealed that aided an understanding of the collagen chemistry. Collagen was found to be rich in proline and hydroxyproline residues, the latter being a relatively rare amino acid. The presence of this amino acid residue in collagen has allowed development of an assay for the concentration of collagen within a tissue (Kivirikko K.I., Laitinen O. and Prockup D.J., 1967). 4-hydroxyproline is almost exclusively present in collagen, where it is formed by post-translational modification of proline. 3-hydroxyproline is found to a lesser extent. Minor amounts are present in other proteins e.g. acetylcholinesterase, elastin, complement C1q and macrophage scavanger receptor (Reid K.B.M., 1974), (Kodama T. *et al*, 1990).

Piez *et al*, showed type I collagen to be a heterotrimer consisting of two types of polypeptide chain termed α1 and α2. These were found in the ratio 2:1 (Piez K.A., Eigner E.A. and Lewis M.S., 1963). This ratio was demonstrated by the introduction of intramolecular crosslinks, the subsequent separation of complexes formed and their amino acid analysis (Nold J.G., Kang A.H. and Gross J., 1970). Advances in sequencing technology allowed the primary structure of the type I collagen α1 chain to be determined. This was a composite sequence from rat and calf skins (Hulmes D.J.S. *et al,* 1973), (Fietzek P.P. and Kuhn K., 1975). A larger number of sequences have now been obtained from α1 and α2 chains in different species. The sequence of

type I collagen α1 chain within an organism is the same in different tissues (Bornstein P., 1969). A greater degree of homology was found between type I collagen α1 chains between species than between α1 and α2 chains in the same organism (Weiss J.B. and Jayson M.I.V., 1982).

Both α1 and α2 chains have three distinct regions. Each has a N terminal region that contains no internal periodicity, and a core region of 1014 amino acid residues containing the repeating tripeptide unit -GLY-X-Y-. It is capped at the C terminus by another aperiodic sequence. The lengths of the α1 and α2 N termini regions are 16 and 9 residues respectively. Those of the C termini are 25 and 6 residues respectively. Various features can be related from the primary structure to the conformation of the macromolecule and its ability to interact with other macromolecules.

The -GLY-X-Y- motif is essential for the formation of the triple helical structure (Rich A. and Crick F.H.C., 1955). Conformational restriction imposed by amino acids in this sequence is essential for the formation of a triple helical molecule. This is opposed to allowing a more entropically stable state to occur. The core region adjacent to the C terminal region has a relatively high proportion of the tripeptide -GLY-PRO-HYP-. This is believed to be a tripeptide unit which confers a great deal of stability to the triple helix. Other regions of the core contain few if any imino acids in the tripeptide sequences. These sequences are therefore thought to be less stable (Fietzek P.P. and Kuhn K., 1975).

The distribution of charged amino acids along the α1 and α2 chains was first revealed using the technique of electron microscopy. Negatively charged

heavy atom stains such as phosphotungstic acid, and positively charged heavy atom stains such as uranyl acetate were used. Electrostatic binding of the stain to various regions of the collagen fibril indicated the position of charged residues. The distribution is known to vary throughout the length of the molecule and is thought to be related to the ability of individual collagen molecules to assemble and form an organised fibrous system.

Very few loci have been found at which a positive or negative charge predominates. Charged regions of the molecule tend to have a balanced amount of negative and positive side chains. The polar and hydrophobic residues are also found in discrete bands in the sequence. These are postulated to contribute to the interactions between laterally adjacent collagen molecules (Fraser R.D.B. and Trus B.L., 1986).

Collagen contains specific glycosylation sites. The amount and position of glycosylation varies between tissues. There is thought to be one primary enzyme directed glycosylation site on type I (α1) chains of rat tail tendon. It has been suggested that the position of this bulky side chain has a role in the packing of collagen. The supramolecular arrangement may be directed by the carbohydrate requiring a specific packing stereochemistry (Morgan P.H. *et al*, 1970).

2.3.2 Physical and mechanical properties of collagen.

The complete triplet of type I collagen can be described as a 285kDal protein, approximately 300nm long and 1.5nm in diameter. Physical methods showed that in solution the molecule acted as a rigid rod (Boedker H. and Doty P., 1956). More detailed studies were subsequently undertaken (Gelman R.A. and Piez K.A., 1980), suggesting that the molecule is semiflexible. A flexible

site in the triple helical region of type I collagen was found by analysis of electronmicrographs. This corresponds to a section of the α1 chains which lacks the amino acid residue proline (Hofmann H. *et al*, 1984). A direct visualisation of the individual collagen molecule's shape was observed (Hall C.E. and Doty P., 1958).

2.3.3 Structure of the triple helix.

The helical nature of collagen was elucidated using a combination of techniques. The unusual amino acid sequence of the helical domain, with its repeating triplet structure, had to be accounted for in any proposed molecular structure. High angle X-ray diffraction and model building were valuable tools in the elucidation of the structural parameters.

Normally, direct structure determination from fibre diagrams is impossible. Structure determination results from model building using the intensity and position of layer lines from fibre diagrams to test the proposed structures. The theory of helical diffraction is outlined by Cochran *et al*, (Cochran W., Crick F.H.C. and Vand V., 1952).

The first proposal that collagen had a triple helical structure was made by Ramachandran, (Ramachandran G.N. and Kartha K., 1954). Advances on the model proposed by Ramachandran were made in 1955 (Rich A. and Crick F.H.C., 1955). This model was able to explain the high occurrence of glycine in collagen. Glycine is the only amino acid which is small enough to fit in the inside of the helix. The left handed nature of the helical turn in each chain is thought to be due to the presence of proline. Hydroxyproline residues are thought to play a role in stabilising the triple helix by forming a system of hydrogen bonds.

A refined model of the triple helical structure that incorporated the right handed supercoil was proposed by Fraser, (Fraser R.D.B., MacRae T.P. and Suzuki E., 1979). A slightly different structure of the collagen triple helix was proposed in 1981 by Okuyama, (Okuyama K. *et al*, 1981). This was based on crystallographic data obtained from relatively short decapeptides of collagen type sequences. A review of proposed models for the collagen triple helical structures has been conducted (Ramachandran G.N., 1988).

2.3.4 The fibrillar nature of collagen.

The nature of collagen as a biomaterial makes the understanding and investigation of the interaction of this molecule with others essential. Structural investigation of the fibrillar nature of type I collagen from rat tail tendon has been conducted mainly by the use of electron microscopy, neutron diffraction and X-ray diffraction. These techniques have allowed the elucidation of a number of parameters concerning the native axial and lateral structure of packing in tendon.

2.3.5 Axial structure.

The axial structure refers to the molecular structure of the fibril projected on to the fibril axis. It disregards the lateral packing of the molecules in the other two directions. Historically, the first visualisation of axial fibrillar structure was obtained by Schmitt *et al*, using electron microscopy (Schmitt F.O., Hall C.E. and Jakus M.A., 1942).

Current concepts of the structure of collagen fibrils derive from the Hodge/ Petruska model (Hodge A.J. and Petruska J.A., 1963). In this model, fibrils are comprised of rod shaped collagen molecules. These are packed in parallel,

and are longitudinally staggered with respect to one another by some multiple of the fibril axis repeat distance (67nm (D)). The length of the collagen molecule is 4.4 D, therefore this arrangement creates alternate regions of high (overlap) and low (gap) density along the direction of the fibril axis.

X-ray fibre diffraction analysis has contributed a great deal to the understanding of the axial structure of collagen, and is a major component of this thesis. Low angle X-ray diffraction studies suggested an axial periodicity (D) of 64nm in dried samples (Bear R.S., 1944). Tomlin and Worthington, using X-ray fibre diffraction, also predicted that a gap/overlap of electron density existed in native collagen fibres (Tomlin S.G. and Worthington C.R., 1956). The advantage of X-ray diffraction is that the technique allows the fibres to be studied in a state pertaining to that *in vivo*. This is almost impossible in electron microscopy when shrinkage of the axial repeat of collagen (D) from 67nm to 64nm occurs during preparation. The use of fixatives such as glutaraldehyde have also been shown to alter the native conformation of collagen (Meek K.M. *et al,* 1982). X-ray diffraction allows a more detailed investigation of the axial structure. The resolution which can be obtained using the electron microscope to study biological specimens is less than that obtained in X-ray analysis. The limit of resolution is ultimately due to the wavelength of electrons and X-rays.

X-ray diffraction has been used to investigate the axial packing of collagen in tendon. The data obtained from diffraction experiments has been combined with other experimental data in a number of ways in order to determine the axial packing density of the collagen fibril. For example, X-ray diffraction data corresponding to a resolution of 1.6nm was combined with phases produced by model building techniques, (Hulmes D.J.S. *et al*, 1977). This

allowed the number of amino acids in a D period to be determined, and also allowed the conformation of the telopeptide regions to be investigated.

The development of the specific heavy atom staining of collagen at relatively few locations, allowed the axial density profile of collagen to be determined by more conventional crystallographic procedures. In this case a resolution of 1.1nm was obtained (Bradshaw J.P., Miller A. and Wess T.J., 1989). Over 100 meridional reflections of collagen have been observed corresponding to the axial electron density of collagen. Therefore the potential resolution of collagen axial structure could be less than 0.67nm. This corresponds to a translation of two amino acids along the triple helical axis in a D repeat.

2.3.6 Three dimensional structures.

X-ray diffraction of collagen has also revealed that collagen can have an ordered three dimensional structure in certain tissues. This was first identified by North, (North A.C.T., Cowan P.M. and Randall J.T., 1954). The majority of research into the three dimensional packing of collagen has been conducted using rat tail tendon. The predominant collagen in this tissue is type I. Rat tail tendon exhibits the greatest amount of three dimensional ordering. Lateral ordering of collagen fibrils has also been found in calcified tissue such as turkey leg tendon (Jesoir J.C., Miller A. and Berthet-Colominas C., 1980). Lamprey notochord, a structure that predominantly contains type II collagen, also exhibits organised packing in three dimensions (Eikenberry E.F. *et al*, 1984). Many tissues do not present evidence of having a highly structured three dimensional structure. The packing of fibres in these structures can be apparently random, or conform to a simple hexagonal close packing scheme.

Until recently, two schemes of collagen packing have been proposed. The microfibril model is based upon observations of discrete fibril size. This implies that fibrils are made from bunches of microfibrils. Microfibrils are made from 5 collagen molecules arranged on a pentagonal basis, staggered axially relative to one another by multiples of D. Packing of microfibrils would give discrete Bragg peaks corresponding to three dimensional packing (Smith J.W., 1968).

The triclinic unit cell model proposes that collagen molecules exist in tendon as microcrystalline regions. The collagen molecule can therefore be represented by a unit cell which has the same structure in each crystallite. The random arrangement of crystallites accounts for the symmetrical nature of the diffraction images. In this scheme, the conformation of type I collagen molecules has been refined using a linked atom least squares procedure in conjunction with high quality X-ray diffraction data. It has been illustrated that the highly ordered overlap region of collagen fibrils consists of a crystalline array of molecular segments inclined by a small angle with respect to the fibril axis. In contrast the gap region is less well ordered and contains segments that are likely to be inclined by a similar angle but in a different vertical plane to that found in the overlap region (Fraser R.D.B., MacRae T.P., and Miller A., 1987). The collagen molecule thus has a D-periodic crimp, in addition to the macroscopic crimp observed visually in the collagen fibres of many connective tissues (Parry D.A.D., 1988). A third scheme of collagen packing has been suggested (Kajava A.V., 1991). In this the collagen molecules pack laterally in a crystalline manner. However the size of the unit cell, and the nature of microfibrils that comprise the unit cell is radically different. The agreement of this structure with observed data is less than that obtained with the triclinic unit cell model.

The packing of collagen in less well ordered systems deserves some attention. Tissues that contain type III collagen generally have a predominant content of type I collagen. The heterofibrils of type I and type III collagen seem to have different structural properties to those of type I collagen only. This is reflected in the proposed helical nature of packing of collagen molecules in fibrils. This gives an apparent reduction in D period value, from 67nm to 65 nm (Brodsky B., Eikenberry E.F. and Cassidy K., 1980). Marchini has reported an angle near 18 degrees for the tilting of individual molecules relative to the fibril axis (Marchini M. *et al*, 1986).

2.4 Crosslinking in collagen.

Crosslinkage in collagen is based on aldehyde formation from lysine or hydroxylysine residues. By stabilising the molecular arrangement within collagen fibrils, intermolecular crosslinks confer stability to the collagen containing tissue.

2.4.1 Reducible crosslink formation.

After secretion from the cell and removal of the procollagen extension peptides by extracellular peptidases, the collagen molecules aggregate in a highly specific manner to form precipitated fibrils. This then acts as a substrate for the enzyme lysyl oxidase (protein lysine 6-oxidase, E.C. 1.2.3.13). This enzyme requires a copper ion and an aromatic carbonyl, pyridoxal 5' phosphate or pyrroloquinoline. The exact nature of the aromatic cofactor depends on the source of the enzyme. The enzyme acts on specific lysine and hydroxylysine residues at the ε- amino group. The oxidative deamination of these residues produces aldehydes. Lysine and hydroxylysine are deaminated to produce allysine and hydroxyallysine respectively. The aldehyde is then free

to react spontaneously in a variety of ways. The reaction between two allysine residues results in formation of an allysine aldol crosslink. This constitutes the intra molecular type crosslinkage which occurs within a single collagen triplet (Bornstein P. and Piez K.A., 1966).

The reaction of either type of aldehyde with the ε- amino group of lysine or hydroxylysine results in the production of reducible intermolecular crosslinks. Initially all possible reactions produce a Schiff base type crosslink. This is also known as an aldimine type linkage. Both the reaction of allysine with hydroxylysine, or the reaction of hydroxyallysine with lysine, allow production of the aldimine dehydrohydroxylysinonorleucine (HLNL). The reaction of lysine with allysine to produce dehydrolysinonorleucine is not regarded as a major crosslink. This is because hydroxylysine, or hydroxyallysine is usually involved in crosslink formation. The reaction of hydroxyallysine with hydroxylysine produces an initial aldimine crosslink dihydroxylysinonorleucine. This can undergo a further spontaneous reaction to form a ketoimine (oxo-imine) type structure *in vivo* (Robins S.P., Shimokomaki M. and Bailey A.J., 1973). This is an Amadori rearrangement, the resultant ketoimine being hydroxylysino-5-ketonorleucine (HLONL). It is the α-hydroxy group of these crosslinks which enables these aldimines to spontaneously rearrange and stabilise as ketoimine structures. This reaction is essentially complete for dehydrodihydroxylysinonorleucine. The ability for the crosslink HLNL to form the acid stable ketoimine, depends on whether it was derived from allysine and hydroxylysine, or hydroxyallysine and lysine. The former being unable to form the ketoimine, the latter is able to do this. The two types of crosslink can be distinguished (after reduction with borohydride) by their reactivity with periodate (Robins S.P. and Bailey A.J., 1975).

The initial aldimine and ketoimine crosslinks are reducible using reagents such as sodium borohydride. The use of tritiated borohydride facilitated the identification of these crosslinks in tissue hydrolysates (Robins S.P., Shimokomaki M. and Bailey A.J., 1973). The use of reductants also allowed stabilisation of acid labile aldimine bonds. The ketoimine type crosslink is stable to weak acids.

2.4.2. Maturation of crosslinks.

The amount of reducible and acid labile crosslinks decreases with the maturation of a tissue (Robins S.P., Shimokomaki M. and Bailey A.J., 1973). The maturation of collagen crosslinks is complex. The large number of tissues containing potential mature crosslinks, and the possible production of artifacts in isolation of putative crosslinks, has complicated a potentially less complex situation. There is now some degree of consensus in the identification of various mature crosslinks and their formation.

Two pathways of mature crosslink production have been proposed. Those which follow the allysine based pathway, and those which follow the hydroxyallysine pathway. In a tissue, this is dictated by the predominant nature of the initial immature reducible crosslink. The allysine route to the production of crosslinks predominates in skin (Mechanic G.L., Gallop P.M. and Tanzer M.L., 1971). The hydroxyallysine route predominates in bone and cartilage (Davis N.R. and Bailey A.J., 1971). Tendon collagen is thought to have a combination of the two types of crosslinks.

In the hydroxyallysine based pathway, a mature crosslink has been identified. This is a trivalent amino acid based on a 3-hydroxypyridinium ring. An initial study identified pyridinoline in Achilles tendon collagen (Fujimoto D., Akiba

K., and Nakamura N., 1977). A further study isolated the crosslink on a large scale from bovine bone (Fujimoto D. *et al*, 1978). Two chemical forms of the 3-hydroxypyridinium based crosslink have been identified. These are, hydroxylysyl pyridinoline (H.P.), encompassing three residues of hydroxylysine, and lysyl pyridinoline (L.P.), encompassing two residues of hyroxylysine and one of lysine (Eyre D.R. and Oguchi H., 1980). The L.P. form is found primarily in bone collagen (Eyre D.R., Koob T.J. and Van Ness K.P., 1984). These crosslinks are prominent in load bearing tendons and their abundance appears to be related to the amount of load the tissue bears. H.P. is regarded as the predominant crosslink of cartilage. Both types of crosslink are completely absent in skin (Moriguchi T. and Fujimoto D., 1978). The photolabile nature of the ring structure may account for this. Skin tissue may have evolved an alternative type of crosslink maturation. Methods of L.P. and H.P. formation have been suggested. It has been proposed that the mature crosslink results from two reducible crosslinks (Eyre D.R. and Oguchi H., 1980). It has also been proposed that the crosslink is formed from a ketoimine crosslink and an allysine residue (to form L.P.) or a hydroxyallysine residue (to form H.P.) (Robins S.P. and Duncan A., 1983).

The maturation products of the allysine pathway are less well understood and conclusions are controversial. Histidine is thought to play a role in the maturation of crosslinks on this pathway. A number of more complex tri- and tetra- functional crosslinks have been identified. Allysine aldol crosslinks can react with hydroxylysine or histidine to form hydroxymerodesmosine and aldolhistidine. These are both reducible, acid labile crosslinks (Tanzer M.L., 1973). Either of these could serve as the precursor for the tetra functional histidinohydroxymerodesmosine (HHMD). These crosslinks are reducible. Since the amount of reducible crosslinks decreases with maturation, these

compounds are unlikely to be the maturation products of this pathway.

Histidinohydroxylysinonorleucine is a stable non-reducible trifunctional crosslink formed by the condensation of HLNL and histidine (Yamauchi M. *et al*, 1987). This has been proposed as the mature product of the allysine pathway in skin.

An alternative view of crosslink maturation has been proposed. A crosslink, so far called compound M, has been isolated from specific CNBr digestion products of collagen. The polymeric material poly α1CB6 contains mature crosslinks which hold the collagen fragments together. This material was found to contain no pyridinoline based crosslinks. The crosslink was found to incorporate lysine residues, and has a molecular mass of 446. A direct correlation was found between the decrease in the quantity of the bifunctional aldimine crosslinks and the production of compound M. It is implied that Compound M results from maturation of the ketoimine crosslinks. This suggests that a common mechanism for the maturation of all collagens exists (Barnard K. *et al,* 1987). Reviews of crosslinking in collagen have been presented (Ricard-Blum S. and Ville G., 1989), (Last J.A., Armstrong L.G. and Reiser K.M., 1990). Diagrams of collagen crosslink type and possible pathways of crosslink maturation are shown in Figure 2.1.

2.4.3. Location of crosslinks.

The amino acid residues which are involved in crosslinking are highly specific (Eyre D.R., 1987). Within the fibrils, the molecules are apparently crosslinked end to end. More complex interfibrillar crosslinking must also occur in the formation of fibres and fibre bundles. Crosslinks in collagen are essential for the maintenance of the strength of healthy tissue, and as such the

nature and the positions of these crosslinks has long been a subject for debate. The approximate positions of several crosslinks have been determined. This is usually carried out by examination of CNBr digests of collagen reduced by tritiated borohydride. One of the first crosslink peptides to be isolated utilising this method was from borohydride reduced cartilage (Miller E.J., 1971). Harding described the isolation of a crosslink peptide from CNBr digests of bovine corneal collagen (Harding J.J., 1978).

One study has approached the problem of examining the nature and location of crosslinks in a non-destructive manner. Neutron diffraction of collagen fibrils specifically deuterated at ketoimine and aldimine groups was conducted. The location of the crosslinkage sites with respect to the fibril structure was determined. It has been proposed that the major sites of crosslinkage exist in the N and C terminal areas of the fibril (Wess T.J., Miller A. and Bradshaw J.P., 1990).

Type I collagen from rat tail tendon is reported to contain allysine derived crosslinks (Bailey A.J. and Peach C.M., 1968). More recent studies have proposed the existence of hydroxylysine mediated crosslinks, (Nakamura Y., 1987). Generally, there are crosslinks between residues in laterally adjacent telopeptides. There is also evidence of another crosslink between a residue in the telopeptide and a residue in the main triple helix of the molecule (Barnard K. *et al*, 1987). More complex crosslinks have been determined in skin collagen (Yamauchi M. *et al*, 1987). Until now, the bifunctional crosslinks which have been observed in type I collagen from rat tail tendon have mostly been proposed to be involved in the role of stabilising sheets of collagen molecules (Miller A., 1982). Collagen exists in a more complex three dimensional structure in most connective tissues (Fraser R.D.B., MacRae T.P.

and Miller A., 1987). Crosslinks have been observed which support three dimensional packing in periodontal ligament (Yamauchi M., Katz P.Z. and Mechanic G.L., 1986). Stereospecific three dimensional packing of collagen has also been implicated in fetal bovine bone (Yamauchi M. *et al,* 1989).

2.4.4. Crosslink related diseases.

The role of crosslinking in collagen is in the maintenance of the structural integrity and strength of a tissue. This is particularly well demonstrated in the condition known as Ehlers-Danlos syndrome. Ehlers-Danlos manifests as hyperextensibilty and hyperflexibility of skin and joints. This syndrome is caused in the majority of cases, by a complete lack of the enzyme lysyl oxidase. Subjects with this condition have low levels of crosslinked collagen and hence reduced strength of their connective tissue.

Studies have examined the relationship between collagen crosslinking and skin strength in Ehlers-Danlos syndrome patients. The biochemical properties of the skin, together with the ratios of type I:type III and the pattern of reducible collagen crosslinks were determined. Ten patients with Ehlers-Danlos syndrome type III, and ten age and sex matched controls were examined. This study illustrated that the Ehlers-Danlos subjects had marked reductions in skin strength and stiffness (42% compared with 22%). The skin thickness, collagen content and ratio of type I:type III collagen were all similar to controls. This illustrated that the changes which are apparent between the two groups may be attributable to the molecular structure of the mature collagen (Kalund S. *et al*, 1990).

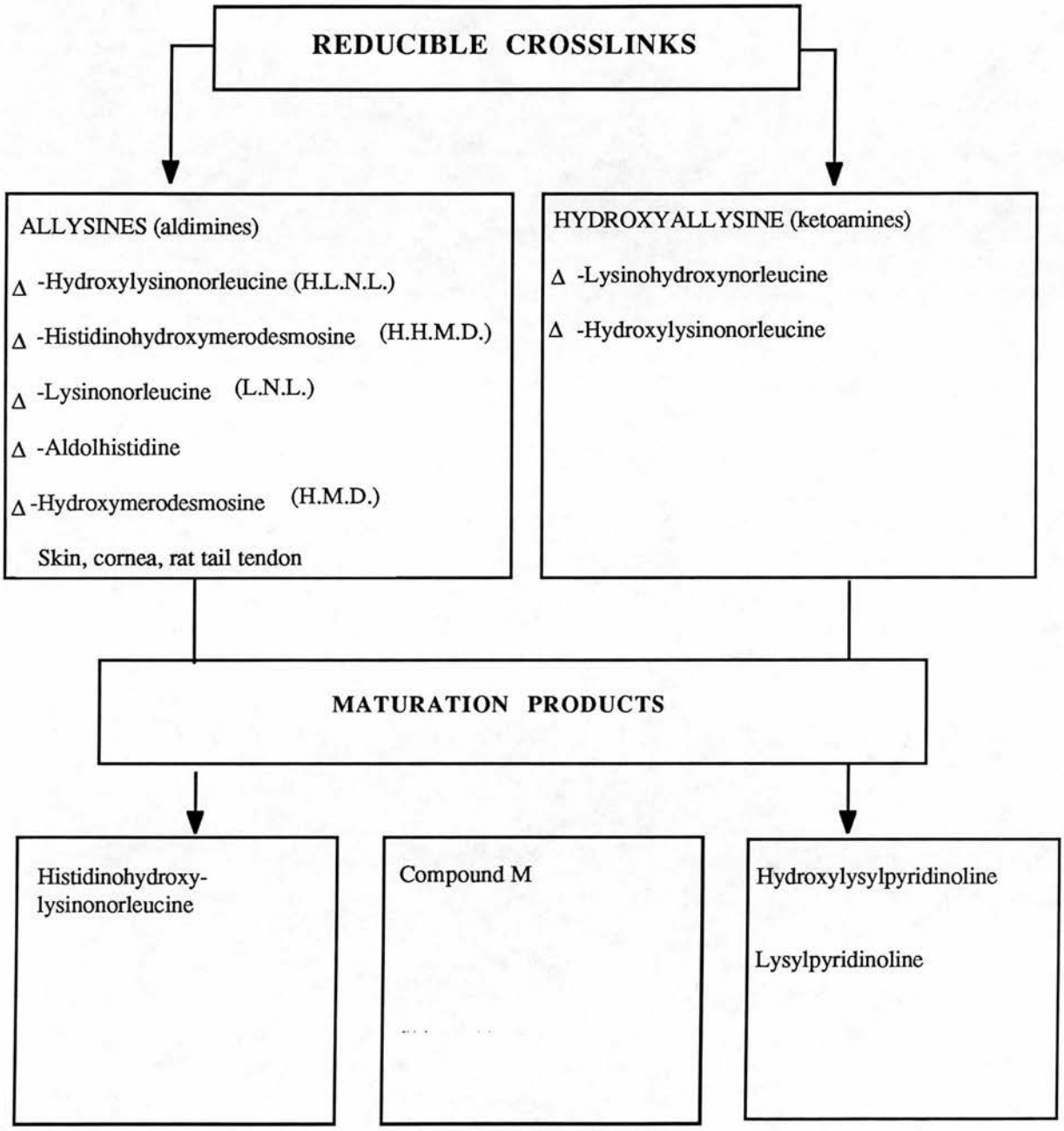


Figure 2.1 : This diagram represents some of the crosslinks which can be found in collagen. The prefix Δ for dehydro signifies the natural, aldimine form of the compounds.

Figure 2.2: This diagram illustrates the formation and the structures of the crosslinks of the allysine and hydroxyallysine pathways. The prefix Δ for dehydro signifies the natural, aldimine forms of the compounds.

2.5 Glycation of collagen.

As people age their cells and tissues change in ways that lead to the body's deterioration and eventually to death. The cells generally become less efficient and less able to replace damaged materials. This change is also associated with stiffening of the tissues. This is evident in the heart muscles and lung tissue, which have been observed to expand less efficiently in the aged. This phenomenon is also observed in ligaments and tendon which have reduced viscoelasticity and flexibility. Elderly people also have a greater chance of developing cataracts and atherosclerosis than their younger counterparts.

It has been proposed that sugar molecules may have a role in the aging process. Accumulation of covalently linked sugar molecules and related crosslinking products is found on a variety of molecules with aging. The effects of diabetes and high blood glucose concentration, produce similar biomechanical problems to those found in aging, but earlier in life (Hamlin C.R., Kohn R.R., and Luschin J.H., 1975). Reduced solubility of collagen with age could be related to the accumulation of glycation products in a tissue.

Molecules such as collagen have been shown to contain non enzymatically attached sugar molecules. This process will henceforth be called glycation. This was demonstrated by the isolation and identification of reducible lysine-carbohydrate condensation products from mature bovine skin (Robins S.P. and Bailey A.J., 1972). The initial reducible adduct of a sugar with a lysine, or hydroxylysine residue is a Ne-(hydroxy)lysine-glycosylamine. This is in equilibrium with a Schiff base linkage between an amide group and a sugar.

The potential of a sugar to undergo glycation with an ε-amine is thought to depend on the propensity of the sugar to be present in the open chain form. The closed ring hemiacetyl forms of sugars are regarded as being unreactive

with respect to glycation. A hexose sugar such as glucose is almost exclusively found in the hemiacetyl form (only 0.002% being in free aldehyde form). Isomers of glucose such as galactose are more commonly found in the open chain reactive form (0.02%). Charged sugar residues such as phosphorylated derivatives e.g. glucose-6-phosphate are mostly present in the open chain form.

Pentose sugars are most commonly found in the open ring structure due to torsional forces restricting ring structure. Sugars with fewer than five constituent carbon atoms tend to be exclusively in the open chain form. This information has been used to assess the relevance of different sugars in an *in vivo* situation (Bunn H.F. and Higgins P.J., 1981).

In vitro experiments have used a variety of short chain or charged amino acids in an attempt to mimic glycation of specific tissues. This allows the effects of glycation to be observed in days or weeks compared to the normal aging process (Tanaka S. *et al*, 1988).

The reaction of sugars with the ε-amine group of (hydroxy) lysine does not necessarily stop with the formation of the Schiff base. The reversible nature of the Schiff base allows dissociation of the sugar adduct. As in the case of some lysyl oxidase based crosslinks, the Schiff base linkage can undergo an Amadori rearrangement into a more stable ketoimine linkage. This structure is thought to be more stable if the initial adduct is between a sugar and a hydroxylysine residue (Perejda A.J. *et al*, 1984).

The reaction between sugars and biological macromolecules has been recognised for a long time (Maillard L.C., 1912). The reaction between sugar

and protein leads to browning of the protein. Chromophores and fluorophores related to the reaction of sugars are regarded as the advanced products of glycation. The process of advanced glycation also leads to crosslinking of proteins such as collagen. This results in decreased digestibility of collagen by proteolytic enzymes (Hamlin C.R., Luschin J.H. and Kohn R.R., 1978). A decrease in solubility in weak acids has also been determined (Schnider S.L. and Kohn R.R., 1981). *In vitro* evidence for crosslink production mediated through glucose has been presented (Kent M.J.C., Light N.D. and Bailey A.J., 1985).

The nature of the sugars which interact with protein *in vivo* is not well understood. Glucose is abundant in mammals, however it does not behave very reactively with respect to crosslinking. Pentose sugars have been proposed as the origin of glycation products. The relatively low levels of pentoses in interstitial fluids may be compensated for by their potential reactivity. The role of ascorbate, threose and other sugars in glycation has not been ruled out (Dyer D.G. *et al*, 1991). The potential reactivity of hexose sugars may increase after initial aldimine formation by oxidative sugar fragmentation. The nature and formation of the crosslinking advanced glycation (Maillard) end products is a matter of debate. It is possible that Amadori products are spontaneously fragmented into dicarbonyl compounds known as deoxyglucosones or ketoaldehydes. These can react with amino groups or arginine residues of proteins to form crosslinks (Farmar J., Ulrich P. and Cerami A., 1988). The reaction of two Amadori products, or the reaction of deoxyglucosone with an Amadori product, can make an advanced glycation end product (Ledl F. and Schleicher E., 1990), (Cerami A., Vlassara H. and Brownlee M., 1988).

A number of glycation end products have been reported. Dyer *et al,* state that the evidence for age dependent accumulation of Maillard products in tissue protein is limited to three compounds (Dyer D.G. *et al,* 1991). Nε(carboxymethyl)lysine is found in lens protein and skin collagen (Dunn J.A. *et al,* 1989). Nε(carboxymethyl)hydroxylysine is found in skin. Pentosidine is found in lens capsule. The carboxymethyl derivatives of lysine and hydroxylysine are the oxidative breakdown products of Amadori rearrangements (Ahmed M.U., Thorpe S.R. and Baynes J.W., 1986). They can serve as indicators of previous glycation events.

Pentosidine is a recently discovered fluorescent crosslink in collagen (Sell D.R. and Monnier V.M., 1989). A compound isolated by Baynes' group, called Maillard fluorescent product 1, was revealed also to be pentosidine. Pentosidine is thought to involve oxidation in its production. Ribose has been proposed as the most likely precursor of pentosidine (Sell D.R. and Monnier V.M., 1990), but other sugars may participate. This crosslink only accounts for less than 1% of the total crosslinks in proteins during browning reactions (Dyer D.G. *et al,* 1991). The role of oxidation in diabetes and age mediated crosslinkage is becoming more important.

Glycation end-products other than those mentioned above have been proposed. The increased fluorescence of aged and diabetic tissue led to a search for potential crosslinks which had similar fluorescence profiles. Pongor *et al,* were the first to suggest a heterocyclic compound generated through the condensation of two Amadori products, 2-(2-furoyl)-4-(5)-(2-furanyl)-1-H-imidazole, (FFI) (Pongor S. *et al,* 1984). This crosslink was however proposed to constitute less than 1% of total advanced glycation end-products. This product was also suggested to be an artifact generated in the acid

hydrolysis of the Amadori product (Njoroge F.G., Fernandes A.A., and Monnier V.M., 1988). Two other pyrrol-related compounds have now been identified. These are 1-alkyl-2-formyl-3,4-diglycosyl-pyrrole (AFGP), (Farmar J.G., Ulrich P.C. and Cerami A., 1988) and 5-hydroxymethyl -1-neopentylpyrrole-2-carbaldehyde pyrraline, this has been found *in vivo* (Hayase F. *et al*, 1989).

Tanaka *et al*, have illustrated that exposure of rat tail tendon collagen to a reducing sugar results in covalent attachment of the sugar to the collagen. X-ray diffraction was used to study the changes in the crystalline unit cell which may have been brought about by glycation of the collagen molecules. A kinetic model describing the process of glycation and subsequent crosslink formation was developed. The results illustrated that glycation resulted in an expansion by more than 12% of the unit cell that describes the three dimensional structure of collagen. This is due to the possible conjugation of adjacent Amadori products, which maintain the amino acids in a rigid extended form. They postulated that this change is similar to the changes which occur in tissue in the natural course of aging (Tanaka S. *et al*, 1988).

2.6 Summary.

The relationship between structure and function proves vital when diseased tissue is involved. This thesis examines the relationship between collagen structure and the diseased state of the colon. The colon wall is rich in fibrous collagen and the study of collagen in both healthy and pathological tissue is the subject of this thesis. The gradual increase of crosslink products over time in organs throughout the body may contribute to age associated morbidity.

Chapter 3

Study of colon collagen.

Materials and methodology.

3.1 A study of the effect of age on the structure of human colonic collagen.

This study was undertaken to determine the effect of age on the collagen of the human colon wall. Samples were taken from cadavers with normal colons and from cadavers shown to have colonic diverticulosis. The potential correlation between age and the condition of the collagen of the colon wall is thought to be important. The condition of certain tissues is known to deteriorate with advancing age. Skin undergoes a very striking alteration with advancing age, as does cornea (Schnider S.L. and Kohn R.R., 1981).

3.2 Tissue collection and analysis.

Colonic specimens were obtained from the *post-mortem* room at the Western General Hospital, Edinburgh, U.K.. Specimens were full thickness and of the dimensions 3cm x 1.5cm. None of the selected subjects died from, or were known to have, colonic disease, other than those identified as having colonic diverticulosis. Specimens were taken from the four defined regions of the colon. These were, ascending, transverse, descending and sigmoid regions. All specimens were obtained prior to 17 hours after death, and within one hour of the *post-mortem* commencing, to reduce autolysis of the tissue. The *post-mortems* were all conducted by the same Consultant Pathologist and the presence or absence of colonic diverticulosis was noted. Twenty five subjects were studied. Twenty were shown to have no evidence of colonic diverticulosis at *post-mortem*. The remaining five were diagnosed at *post-mortem* as having colonic diverticulosis.

The median age of the five colons with colonic diverticulosis was 71 years (range 67-80) and comprised 3 males and 2 females. The median age of the twenty control subjects was 58 (range 20-79) and comprised 10 males and 10 females, see Table 3.1 for the list of subjects.

Specimens of the proportions described above were dissected from the four defined regions of the bowel (see Figure 1.1). These sections were maintained in mammalian buffered Ringer's solution for 20 minutes, before being prepared for further analysis.

3.2.1 Measurement of total collagen content.

Colon tissue was full thickness and was randomly dissected from the colon wall. The analyses were performed on whole tissue from the site specified and not only on localised diseased tissue. The decision was made because this thesis attempted to examine the overall effect of age on the colonic wall collagen and not solely on the affected regions. The resected sections were washed in mammalian buffered Ringer's solution. 1g sections were homogenised using a Polytron homogeniser in 0.5M acetic acid (1ml/100mg wet weight of tissue). The tissue was digested by shaking at 4^0C for 48 hours in 0.5M acetic acid. The resulting homogenate was then centrifuged at 37000g for 60 minutes and the pellet and supernatant removed and stored separately (Chang K. *et al*, 1980).

The supernatant and the pellet were then hydrolysed in 6N HCl for 24 hours at 110^0C in preparation for analysis of the hydroxyproline content. The supernatant was analysed for collagen content by measuring the hydroxyproline content (Kivirikko K.I., Laitinen O. and Prockup D.J., 1967). This fraction was termed acid soluble collagen. The pellet from the extraction was also analysed for hydroxyproline content. This fraction was termed acid insoluble collagen.

The basis for the measurement of hydroxyproline is that, almost all of the hydroxyproline found in animal tissues is found in collagen. The analysis makes use of the fact that hydroxyproline can be easily oxidised. This can then be

converted into a relatively specific chromophore with p-dimethylamino benzaldehyde. All of the materials, including the hydroxyproline used as a standard, were purchased from Sigma Pharmaceuticals, Poole Road, Dorset, England, U.K..

The total collagen content of the tissues was calculated by the addition of the collagen content of the acid soluble and the acid insoluble fractions. Total collagen content is expressed as a percentage of the wet weight of the original tissue. This technique was carried out on all four previously designated regions of the colon in each of the twenty five subjects. Analyses were carried out in triplicate and all included a water blank and hydroxyproline standards.

3.2.2 Measurement of insolubility of collagen.

This technique was used for all four previously designated regions of the colon in each of the twenty five subjects. The ratio of insoluble collagen to soluble collagen, the solubility index, gives an indication of the amount and possibly the nature of crosslinks present. Acid soluble collagen contains aldimine crosslinks (-CHOH-CH=N-). Acid insoluble collagen contains ketoimine crosslinks (-CO-CH_2-NH-), and mature crosslinks of both the allysine and hydroxyallysine pathways. Crosslinks resulting from the Maillard reaction may also be present in the insoluble fraction. The relationship between crosslinkage and collagen solubility has been addressed (Robins S.P., Shimokomaki M., and Bailey A.J. 1973).

PATIENT	AGE	SEX	CAUSE OF DEATH	TIME AFTER DEATH	DIVERTICULOSIS ?
1	20	MALE	Cardiac failure	12 hours	NORMAL
2	30	FEMALE	Ovarian cancer	15 hours	NORMAL
3	36	FEMALE	Ovarian cancer	16.5 hours	NORMAL
4	45	FEMALE	Bronchopneumonia	11 hours	NORMAL
5	59	MALE	Myocardial infarction	16 hours	NORMAL
6	63	FEMALE	Mitral valve disease	14 hours	NORMAL
7	69	FEMALE	Breast cancer	14 hours	DIVERTICULOSIS
8	70	MALE	Myeloma	15 hours	NORMAL
9	73	FEMALE	Cardiac failure	17 hours	DIVERTICULOSIS
10	79	FEMALE	Liver carcinoma	6.5 hours	NORMAL
11	60	MALE	Myocardial infarction	11 hours	NORMAL
12	50	FEMALE	Chronic renal failure	12 hours	NORMAL
13	80	MALE	Myocardial infarction	17 hours	DIVERTICULOSIS
14	38	MALE	Cerebrovascular disease	15 hours	NORMAL
15	54	MALE	Myocardial infarction	13 hours	NORMAL
16	65	FEMALE	Cardiac arrest	15 hours	NORMAL
17	69	MALE	Pulmonary embolism	13 hours	NORMAL
18	65	MALE	Myocardial infarction	7 hours	NORMAL
19	53	FEMALE	Chronic renal failure	14 hours	NORMAL
20	71	MALE	Pulmonary embolism	9 hours	DIVERTICULOSIS
21	43	FEMALE	Trauma (road traffic accident)	6 hours	NORMAL
22	67	MALE	Lung cancer	15 hours	DIVERTICULOSIS
23	70	FEMALE	Myocardial infarction	12 hours	NORMAL
24	63	MALE	Myocardial infarction	10 hours	NORMAL
25	57	MALE	Myocardial infarction	12.5 hours	NORMAL

Table 3.1 : This table illustrates the subjects from which colonic tissue was removed for this study. The subjects were within the age range 20 to 80 years and were specifically chosen as the cause of death was not related to bowel disease. The cause of death and time after death when the tissue was removed are shown together with the age of the subjects. The presence of colonic diverticulosis is also shown.

3.3 A study of the effect of diet on the structure of colonic collagen using a suitable animal model.

This section of the thesis was initiated by a study by Fisher*et al.* They designed a large scale animal experiment to study the effect of diet on the development of colonic diverticulosis (Fisher N. *et al*, 1985). This led to the idea that tissue affected by colonic diverticulosis would be available for biochemical and structural analysis by dietary regulation. This type of study would allow analysis of the theory proposed by Painter, relating the dietary fibre intake to the development of colonic diverticulosis (Painter N.S., 1975). Diet has a profound effect on the function of the colon. It would seem reasonable to assume that there would be a parallel effect on the structure of the colon wall to accommodate colonic function. There may be a change in the distribution or relative proportion of the components of the colon wall. All of these changes would have a profound influence on the structure, and hence the function of the colon wall. The primary effect of dietary fibre on the colon is to promote reduced transit times (increased propulsive activity in the colon) and faecal bulking (reduced colonic water absorption). Both of these effects would influence the function and the structure of the colon wall. It is thought that a lifelong deficiency of dietary fibre would have an important influence on the eventual composition and structure of the colon wall. If this was the case, both the dietary fibre theory (Painter N.S., 1975) and the structural theory (Morson B.C., 1963) would exist in parallel.

It is thought by some groups that dietary fibre, or lack of dietary fibre, exerts an effect on the colon wall throughout life, and that there are no other influences involved (Burkitt D.P. and Trowell H.C., 1975). Other groups believe that there are social and economical influences associated with dietary composition (Barker D.J.P. and Osmond C., 1987). As the condition is complex and associated only with elderly populations, it would seem reasonable that there

may be more than one factor involved in the aetiology of colonic diverticulosis. Only a percentage of the elderly population is affected by colonic diverticulosis (50% in the ninth decade). It is proposed that the difference between those affected and those with healthy bowels is the diet which they have consumed for a lifetime.

The proposal that collagen of the colon wall could be affected by dietary fibre, may seem a little hypothetical. Connective tissue may be altered simply due to the pressures which are placed upon it. Colonic diverticulosis is associated with very high intraluminal pressures in the colon and this could result in an alteration to the physical nature of the colon wall.

3.3.1 Production of a suitable animal model.

An animal experiment was undertaken in the animal unit of the Western General Hospital, Edinburgh, U.K.. The aim of this experiment was to determine the effect of diet on the colon of laboratory rats after a long term dietary regimen. The reason for this is that for many years prolonged lack of dietary fibre has been a causative agent in colonic diverticulosis. The production of an animal model is necessary, as a long term dietary experiment of this nature carried out on humans would be problematic and unethical. An animal model is the only practical manner to study this problem.

3.3.2 Details of the experiment.

The animal which was chosen for this particular study was the laboratory rat, Wistar variety. The experimental feeding time was designated as 18 months. This period was considered to correspond to the average lifetime of a human. Laboratory rats were bred specifically for this purpose. Uncertainty existed in the design of the experiment as to contamination of the pups feeding during their early stages of life by the maternal diet. This experiment was developed in two

limbs. The pups were fed on either a low or high fibre diet.

3.4 Dietary requirements.

The two diets differed primarily, in the concentration of dietary fibre, and were purchased from Special Diet Services (S.D.S.), Lavendar Mill, Manea, Cambridgeshire, U.K.. The non-starch polysaccharide contents were kindly measured by H. Englyst (Englyst H., Wiggins H.S. and Cummings J.H., 1982).

Experimental (group 1): Low fibre diet fed young, bred from low fibre diet fed parents, **Familial Low Fibre Group. (F.L.F.).**

Experimental (group 2): Low fibre diet fed young, bred from high fibre diet fed parents, **Weaned Low Fibre Group. (W.L.F.).**

Control group (group 3): High fibre diet fed young, bred from high fibre diet fed parents, **Familial High Fibre Group. (F.H.F.)**

3.4.1 Dietary composition.

Low fibre diet		High fibre diet (control)	
Component	**%**	**Component**	**%**
Commercial pregelatinised wheat starch	**71.2**	Crude fibre (Wheat fibre)	**3.6**
Wheat gluten	**8.9**	Crude protein	**18.1**
Casein	**8.0**	Carbohydrate	**66.9**
Dried egg	**4.0**	Crude oil	**2.7**
L-lysine hydrochloride	**0.3**	Leucine	**1.4**
L-threonine	**0.1**	Lysine	**1.0**
Corn oil	**2.0**	Calcium	**0.8**
Vitamin mix	**1.0**	Vitamin mix	**1.0**
Mineral mix	**4.5**	Mineral mix	**4.5**
Supplied in powdered form and then mixed with water to form a paste.		Supplied in pelleted form.	

Table 3.2 : This table illustrates the composition of the low and high fibre diets, in terms of the protein and carbohydrate sources. All values are shown as a percentage of the total. Information from S.D.S., Lavendar Mill, Manea, Cambridgeshire, U.K..

Plate 3.1 : Photograph of the low and high fibre diets to illustrate the different consistencies. The low fibre diet is supplied in a powdered form and mixed with water immediately prior to feeding. The low fibre diet is supplied and fed in pelleted form.

Dietary Composition

Low fibre diet		**High fibre diet**	
Component	%	Component	%
Digestible Carbohydrate	78.4	Digestible Carbohydrate	58.3
Non Starch Polysaccharide	1.7	Non Starch Polysaccharide	13.3
Digestible Protein	12.4	Digestible Protein	20.5
Lipid	2.0	Lipid	2.4
Vitamin mix	1.0	Vitamin mix	1.0
Mineral mix	4.5	Mineral mix	4.5
Metabolisable energy.	**2990Kcal/Kg**	**Metabolisable energy.**	**2855Kcal/kg**

Table 3.3 : This table illustrates a more detailed analysis of the dietary composition of the low and high fibre diets. The values for each of the components are expressed as a percentage of the total. The difference in the digestible carbohydrate content is shown.

3.4.2 Breeding.

The breeding conditions were such that the parents were fed on the appropriate diet for a period of one month prior to mating. This period was considered a suitable adjustment period. The animals each produced relatively large litters (~15 young, consisting approximately 50% male and 50% female). Only the male progeny were required for this study, since this reduces hormonal complications. The female progeny were removed from the parents and sacrificed, in order to allow the males to grow more efficiently. In the case of experimental groups 1 and 2, the young were weaned onto the low fibre diet after 17 days suckling and not exposed to any other source of dietary fibre. The low fibre diet had a predetermined concentration of dietary fibre, which was calculated to represent the relatively low fibre diet consumed by the typical western man.

3.4.3 Caging requirements.

One particular condition of the design of the experiment, was that the rats had to be caged on a bedding of refined cat litter as all other commercial forms of animal bedding contained fibre of some form. The particular cat litter used was Thomas absorbant refined cat litter, which is a clay based, finely chipped gravel. This bedding was chosen as it did not contain dietary fibre at all. This means that animals could eat the bedding without ingesting fibre, which would interfere with the experiment. Gridded cages were considered at the beginning of the experiment. These cages were dismissed as they were thought to cause excess stress to the animal for such a long period of time.

After 17 months duration of the experiment, the bedding had to be changed from commercial cat litter to refined sand as a veterinary requirement. Some of the animals were considered so obese that they were dragging their abdomens on the floor of the cages. This was causing abrasions due to the gravel based cat litter.

Refined sand was considered to be more suitable for the size of the animals than the cat litter. This form of bedding contained no dietary fibre at all. All rats were tail marked with a coloured marker pen. This method of identification was necessary as the rats were weighed at the same point each week to allow a check on the health of the animal. At periodic intervals the rats which were being fed the low fibre diet had to have their teeth cut, as they had no means of wearing down the two incisor teeth.

3.4.4 Faecal collection experiment.

In order to study the influence of the different diets on the function of the colon, a faecal collection experiment was carried out. Faeces were collected over a twenty four hour period from the two experimental groups of rats and the control group. This was carried out to establish any difference in faecal output due to the difference in the dietary fibre content of the diets. Five rats were selected from each group on a weight matched basis. They were placed individually in gridded cages, 24 hours prior to the collection in order to allow a period of adjustment to the new surroundings. They were each supplied with *ad libitum* food of the appropriate diet. Faeces were collected at hourly intervals, from each of the individually caged rats, and weighed wet. They were then taken back to the laboratory, freeze dried and weighed once more.

3.4.5 Measurement of food intake.

In order to correlate any differences in faecal output with diet, the exact amount of food which was taken in must be known. A study was undertaken to measure the food intake of the rats from all three groups. This study involved five rats from each of the groups being caged individually and being fed a weighed amount of diet over a period of twenty four hours. Any remaining food was weighed and subtracted from the initial total. This gives the total amount of food consumed for each rat.

3.5 Tissue collection and analysis.

After 18 months it was decided that the rats were of such an age that maximum experimental benefit should be gained from tissue extraction and analysis. The differences, if any, between the three groups would be evident. The particular method used to kill the rats was overdose by ether. The rats were considered too large to kill by cervical dislocation, the average weight being 700g +/- 256g. Immediately after death the rats were all subjected to a *post-mortem* examination by the Regius Professor of Forensic Medicine, Professor Anthony Bussutil. This was the same pathologist who had carried out the human *post-mortems*. The rat *post-mortems* were carried out to examine the condition of the intestines, and in particular the colon. The presence or absence of diverticulosis was noted.

The *post-mortem* also examined:

(1) the general condition of the body; (2) the heart; (3) the lungs; (4) the pancreas (5) the liver; (6) the skin and (7) the testes.

This was to detect any abnormalities in the form of tumours or lesions.

3.5.1 Further analysis.

The tissues which were removed from the body for further analysis were:

(a) **Small intestine.** This was washed in mammalian Ringer's solution, measured, weighed and retained for collagen measurements.

(b) **Colon.** The colon was removed, washed in mammalian Ringer's solution, measured, weighed and then divided into the defined regions before being retained for collagen analyses. The defined regions were, ascending, transverse, descending and sigmoid as in the human experiment. In addition a section of each of these specimens was fixed and removed for further histological and pathological examination.

(c) **Caecum.** The caecum was removed and washed in mammalian Ringer's solution and then tissue samples were retained for collagen measurements. In addition sections were removed for detailed histological and pathological examination.

(d) **Tumours and any other abnormalities.** These were removed and fixed for further detailed histological and pathological examination.

3.6 Measurement of total collagen content.

Total collagen content of the intestinal contents was carried out as per section 3.2.1, using the method described by Chang *et al*, (Chang K. *et al*, 1980). The tissues which were analysed for total collagen content were: (a) ileum, (b) caecum. (c) ascending colon, (d) transverse colon, (e) descending colon, (f) sigmoid colon. In this analysis 1g wet weight of tissue was used, and the collagen content was expressed as a percentage of this.

3.6.1 Measurement of acid soluble collagen.

The acid solubility of the collagen in the tissues stated above in section 3.6 was measured as per section 3.2.2. The solubility index was expressed as a ratio of insoluble collagen to soluble collagen.

3.7 X-ray fibre diffraction analysis.

This section describes the methodology involved in X-ray fibre diffraction experiments. It includes a wide range of topics including, production of X-rays, sample preparation and methods of data collection and analysis.

3.7.1 Instrumentation and data collection.

The X-ray diffraction data collected and presented in this thesis were produced using the synchrotron source at the Science and Engineering Research Council

(S.E.R.C.), Synchrotron Radiation Source (S.R.S.), Daresbury, England, U.K.. The synchrotron consists essentially of a beam of electrons, orbiting an evacuated ring. Electrons are provided by an injection system, then accelerated and focused by magnetic fields. The effect of angular acceleration of the electron around the ring, at relativistic speeds, results in the production of a wide wavelength band of electromagnetic radiation at a tangent to the electron path.

The S.R.S. was built between 1975 and 1980. It is a 2GeV electron storage ring dedicated to the production of synchrotron radiation. The importance of spectral brilliance (photons s^{-1}, mm^{-1}, $mrad^{-1}$, $s\lambda/\lambda^{-1}$) in experimental investigation, necessitated the upgrading of the synchrotron. This was achieved by the installation of the High Brightness Lattice (H.B.L.) in the main storage ring in 1987. The synchrotron operates at a current of 300mA with two beam injections per day.

The synchrotron consists of:-

3.7.1.1 Linac. The linear accelerator.

This delivers electrons for the synchrotron usually operating at 12MeV. Electrons are released from a heated cathode and accelerated by an anode to a high potential, firstly across a D.C. potential in the electron gun, and then to 12MeV in a S band accelerator system.

3.7.1.2 Booster synchrotron.

The electrons from the accelerator are further boosted by the use of a small ring synchrotron, the booster synchrotron. This provides the beam for injection into the synchrotron storage ring at 600MeV. A transfer path delivers the electrons to the main storage ring.

3.7.1.3 Storage ring.

This controls the electron beam to a small cross sectional area, and the electromagnetic radiation is extracted along beamlines. The storage ring accumulates electrons from the injection system, accelerates them to a peak energy of 2GeV and keeps them in an orbit for approximately ten hours. Quadropole magnets are used to bend the electrons into their almost circular orbit.

The resultant radiation from the synchrotron has a number of properties:-

(a) **High Flux.** The intensity of radiation from the synchrotron is 10-10^7 times brighter than conventional rotating anode X-ray sources.

(b) **Angular divergence.** The angular divergence of the radiation produced is similar to that of a laser.

(c) **Polychromatic radiation.** The electromagnetic radiation is polychromatic and includes wavelengths of 1000-0.001nm, the U.V. to X-ray regions.

The high flux of electromagnetic radiation provided by the synchrotron, enables the diffraction pattern of samples which scatter weakly to be viewed in minutes compared with a matter of days using a conventional source. The effect of beam damage on a sample is directly proportional to the time spent exposed to the X-ray beam together with the amount of flux passing through a sample (Mandelkow E., Mandelkow E.M. and Bordas J., 1981). The relation of a synchrotron diffraction pattern of a sample to the *in vivo* conformation of the molecule is closer than that found using a conventional source.

3.8 Beamlines.

The synchrotron storage ring provides electromagnetic radiation which is released down beamlines. Beamlines are situated at ports around the circumference of the storage ring where radiation is channelled to individual experimental stations. The purpose of this is to provide radiation of the correct

wavelength for varied types of research. In the case of X-rays, a number of beamlines at the S.R.S., Daresbury are dedicated to selecting a narrow range of radiation in the wavelength region corresponding to the CuKα peak of conventional X-ray sources (0.154nm).

Amongst these beamlines are those designated 7.2 and 8.2 non crystalline diffraction (N.C.D.). These beamlines each have special features which make them suitable for certain fields of X-ray experimentation.

The nature of the axial periodicity of collagen is such that the first order of diffraction ($1/67nm^{-1}$) can only be obtained using a camera of sufficient length to separate it from the main beam. The length of camera capable of doing this at the S.R.S., Daresbury is a minimum of 1.5 metres. It is impossible to obtain all of the required reflections from one camera length and therefore two separate beamlines are used. The first 12 orders of diffraction were collected on small angle station 8.2 and orders 9-30 were collected at the beam station 7.2.

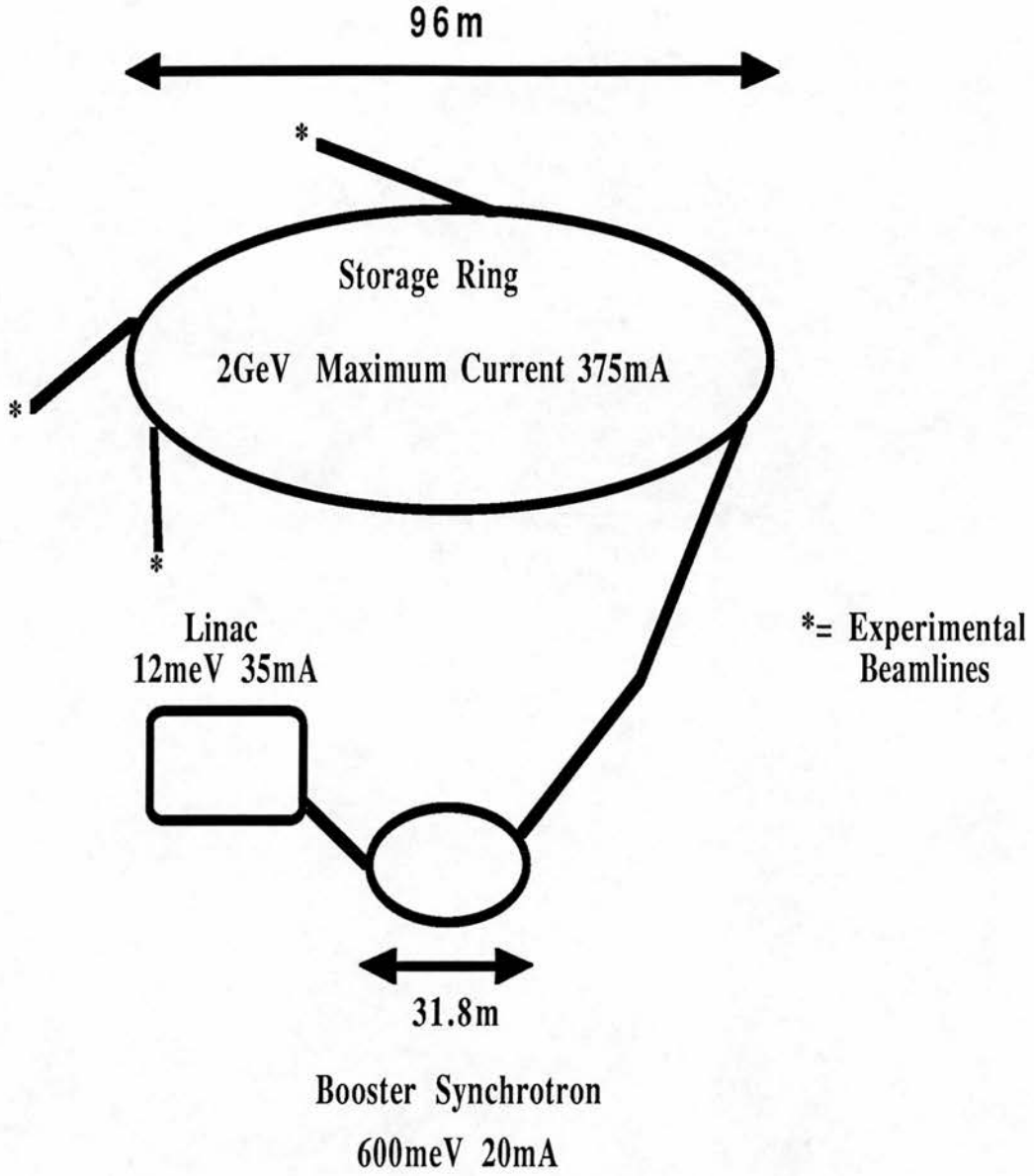


Figure 3.1 : Diagrammatic representation of the S.R.S. at Daresbury, England.

3.8.1 Cameras.

In the work involved in this thesis, the cameras were relatively simple. For small angle experiments, the sample is placed in front of a mica window. The body of the camera consists of an aluminium tube sealed at the other end by X-ray transparent mylar. The mylar must be correctly orientated so as to avoid mylar scatter on the diffraction pattern. Air scatter, which produces a cloud on the film around the main beam is reduced by evacuation of the camera.

For higher angle diffraction experiments the camera on beam station 7.2 N.C.D. is used. The camera length in this case is ~300 mm from collimator to film pack. The body of the camera is made of brass and has an aluminium extension, the whole system being flushed through with helium to reduce the unwanted air scatter. Helium is used since an evacuated camera is less practical in this case as the sample cell is placed inside the camera and the sample cell is not vacuum proof.

For this study, data were collected by the use of a film pack to record the diffraction pattern. The main beam is stopped by a lead cup or beam stop which is shaped to absorb the main X-ray beam and stop scatter onto the film.

3.8.2 Human and animal sample preparation and the sample cell.

One sample cell was designed for use in all of the X-ray experiments and its purpose was to:-

(1) Maintain the sample in a hydrated state,

(2) allow the sample to be positioned correctly in the X-ray beam.

The reason for the first of these properties is that a constant humidity must be maintained. The reason is that the intensities of the meridional and off meridional reflections vary with the humidity around the fibre. A constant relative humidity is maintained by suspension of the sample over a salt solution

of a known concentration (usually saline, 0.15M NaCl). The external temperature around the cell was maintained constant to +/- 2^0C.

3.8.3 Sample preparation.

Human colon collagen samples were prepared by a combination of sharp and blunt dissection as described by Thomson *et al*, in buffered mammalian Ringer's solution (Thomson H.J. *et al*, 1987a). This technique allowed the isolation of the submucosa of the colon which is the most abundant source of collagen fibres. The samples were then maintained in the buffered mammalian Ringer's solution while being tensioned in the sample cell. The sample can be used without deterioration or drying out for up to 4 hours. Tissue was removed from the colon longitudinally, maintaining the approximate direction of the fibres axially for X-ray experiments. The direction of the collagen fibres in the human colon has been described as a lattice of collagen fibres running at an angle of 50^0-55^0 to the longitudinal axis of the colon (Gabella G., 1987). The fibres were aligned axially by stretching the submucosa in the longitudinal direction to a small degree. This allowed a higher degree of axial order to be viewed on the diffraction pattern without damaging the collagen in any way. All of the samples were stretched by the same amount.

The human samples examined in this study fell into three categories:-

(1) Healthy, young individuals - Healthy colon and age range 20 - 45.

(2) Healthy, aged individuals - Healthy colon and age range 45 - 80.

(3) Diseased, aged individuals - Colons affected by colonic diverticulosis and age range 45-80.

A variety of sample exposure times were tested from 20 minutes to 120 minutes for the high angle experiments, and 1 minute to 15 minutes for the small angle experiments. The optimum time used in all of the X-ray experiments outlined in this thesis was 60 minutes for the high angle experiments on beamline 7.2, and 5 minutes for the small angle experiments on beamline 8.2.

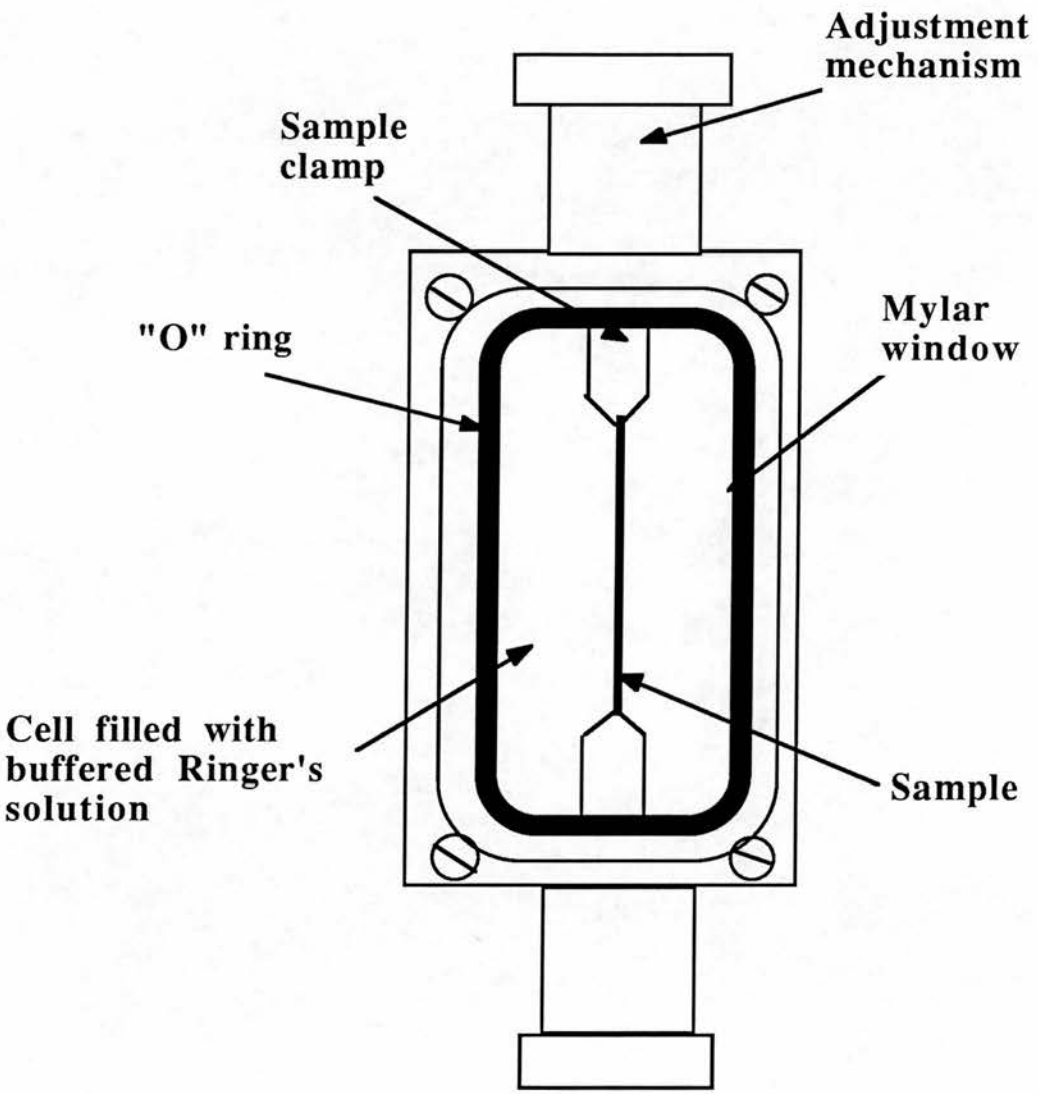


Figure 3.2 : This is a diagrammatic representation of the sample cell which was used in all of the X-ray diffraction experiments presented in this thesis. The essential points of the cell are illustrated, the mylar window, 'O' ring and clamping system to tension the colon collagen.

3.9 Data collection and analysis.

The accuracy of a structure determined by X-ray diffraction depends on the accuracy with which the data are obtained. This encompasses a whole range of topics from the quality and repeatability of sample preparation, to the method of data collection and analysis.

3.9.1 Data collection.

Data collection can take two forms. One is the use of X-ray sensitive film and the other is the use of electronic detectors. In this case the option chosen was to use X-ray sensitive film. The film used here was Ceaverken reflex 25. The range of meridional intensities requires a pack of films (average 5) to be used in each data collection. Each film therefore gives a different intensity dependent on the distance of the film from the sample. The more intense diffraction peaks can be estimated from those films furthest away from the sample without saturation. The less intense diffraction maxima can be observed with accuracy from those films closest to the sample.

3.9.2 Data analysis.

Analysis of the data collected during this thesis would take longer than the time available, and therefore the data will be presented. Presentation of the meridional intensities allows analysis of the primary differences between the three groups of colons previously described. The films were all scanned on a Joyce Loebl Chromoscan 3 densitometer to record the position and amplitude of the diffraction peaks, to determine the intensities of the meridional reflections. The resultant intensities were scaled to remove the background and then scaled to a first order of 10000 to allow analysis of the lower orders of diffraction.

This thesis will attempt to examine the collagen of the submucosa of human colon to the highest resolution to date. Any differences between normal and

pathological tissues will also be examined. The experiments involved in this thesis allow the effects of both age and diet on the collagen of the colon of rats and humans to be determined. The experiments allow analysis of both of the theories pertaining to the development of colonic diverticulosis.

Chapter 4

The effect of age on the structure of human colonic collagen.

4.1 Results of the study of the effect of age on the structure of human colon collagen.

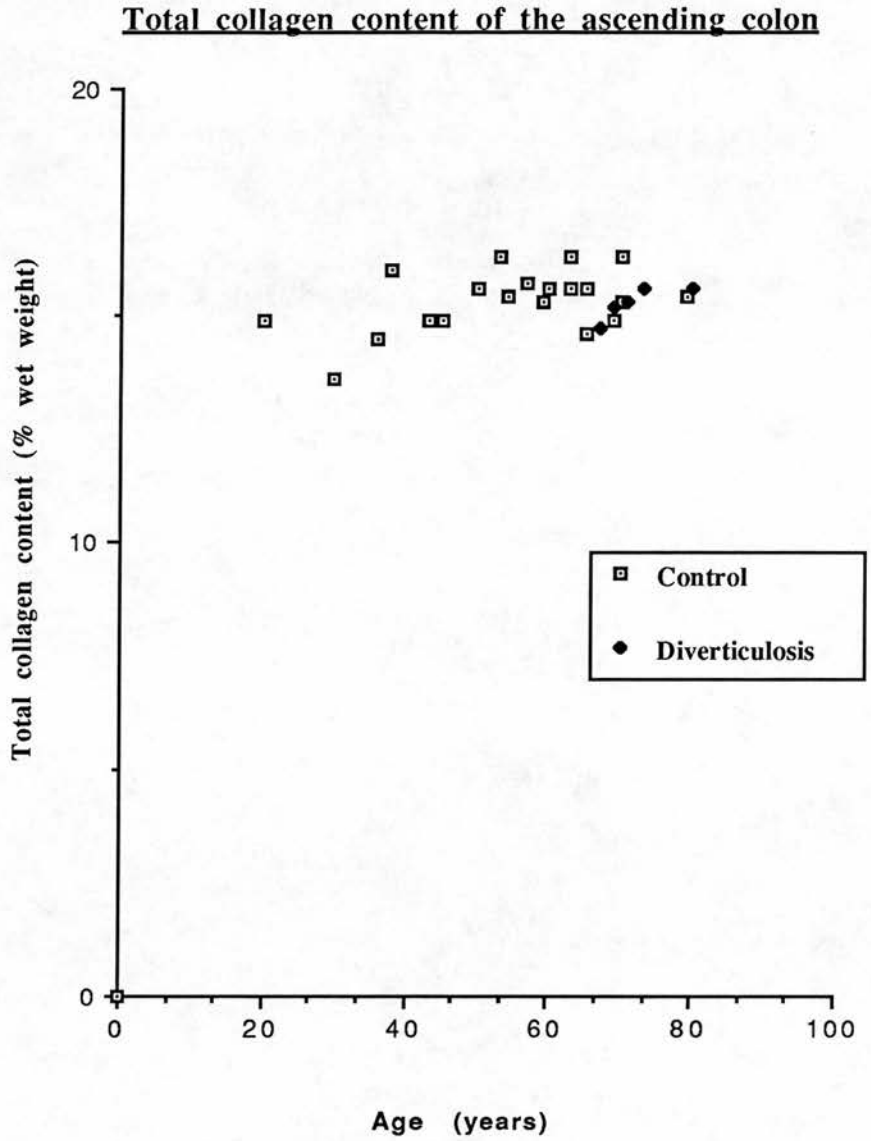
This study was concerned with the analysis of submucosal tissue from the colons of twenty five subjects from the Edinburgh area. All colonic sections were treated using the methodology described in Chapter three. The large age range (20 to 80 years) which was studied allowed measurement of any differences in the collagen, due to aging of the tissue.

4.1.1 Total collagen content.

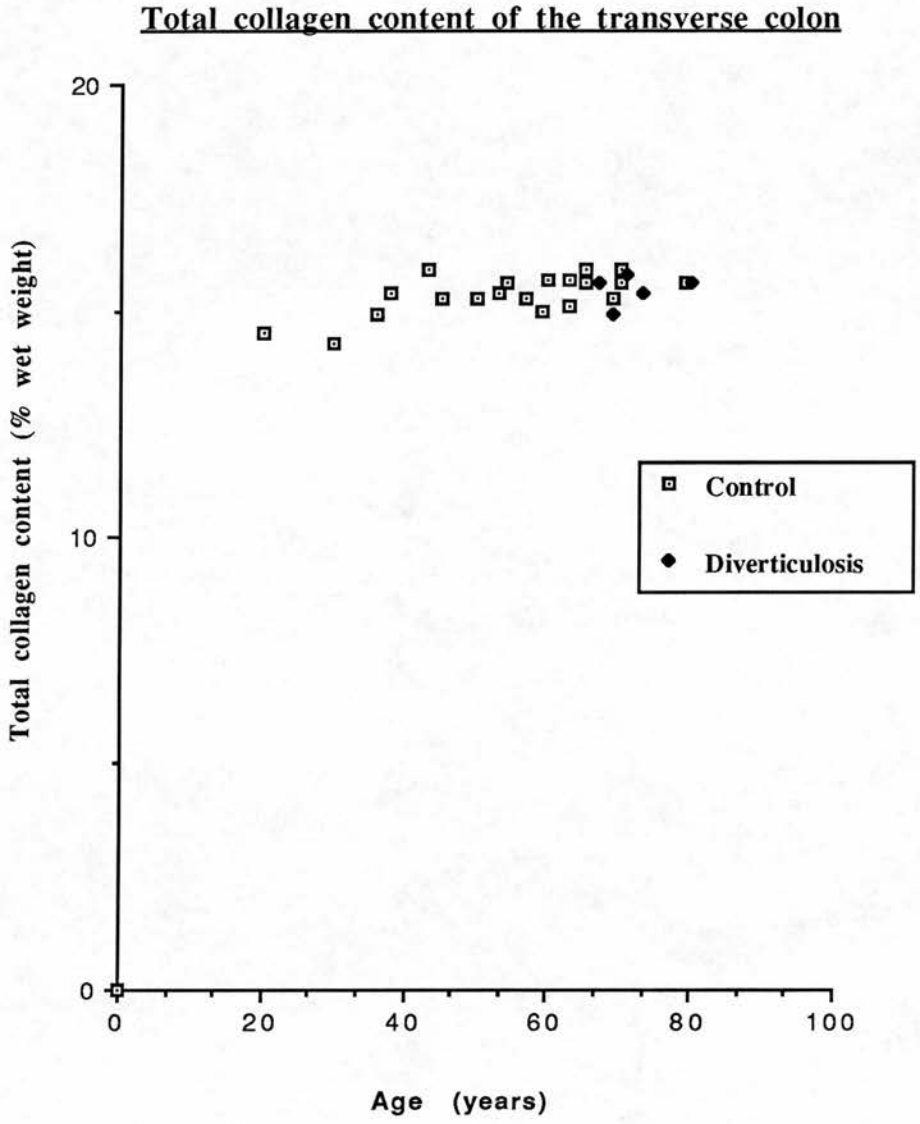
This involved all four colonic sections of the twenty five subjects under study. Results were obtained in triplicate and are expressed as median and range of the three results. The results from this analysis are shown on Table 4.1 and are illustrated graphically on Graphs 4.1-4.4. Statistical analysis of the results is illustrated in Table 4.2.

PATIENT	AGE	SEX	ASC.	TRANS	DESC.	SIGMOID	DIVERTICULOSIS ?
1	20	MALE	14.9 (14.1-15.4)	14.5 (13.9-15.0)	14.6 (13.9-15.2)	14.3 (14.0-15.4)	NORMAL COLON
2	30	FEMALE	13.6 (13.1-14.8)	14.3 (14.0-14.9)	13.7 (13.1-14.7)	13.8 (13.2-14.7)	NORMAL COLON
3	36	FEMALE	14.5 (13.9-14.9)	14.9 (14.1-15.5)	14.6 (13.9-14.9)	14.3 (13.9-14.8)	NORMAL COLON
4	45	FEMALE	14.9 (13.8-15.3)	15.3 (14.7-15.6)	15.0 (14.4-15.9)	15.2 (14.3-15.7)	NORMAL COLON
5	59	MALE	15.3 (14.6-15.8)	15.0 (14.5-15.5)	15.7 (14.9-16.1)	15.9 (15.0-16.4)	NORMAL COLON
6	63	FEMALE	15.6 (14.9-15.9)	15.1 (14.5-15.7)	14.6 (14.2-15.5)	14.9 (14.3-15.4)	NORMAL COLON
7	69	FEMALE	14.8 (14.3-15.0)	14.9 (14.2-15.6)	15.6 (14.1-15.9)	16.0 (15.2-16.4)	DIVERTICULOSIS
8	70	MALE	15.3 (14.9-15.6)	15.9 (14.3-16.1)	16.0 (15.1-16.3)	16.7 (15.3-16.9)	NORMAL COLON
9	73	FEMALE	15.6 (15.0-16.1)	15.4 (14.9-15.7)	15.3 (14.8-15.8)	15.4 (15.0-16.1)	DIVERTICULOSIS
10	79	FEMALE	15.4 (15.0-16.1)	15.6 (14.9-15.9)	14.6 (14.1-15.3)	14.9 (14.1-15.4)	NORMAL COLON
11	60	MALE	15.6 (15.0-15.9)	15.7 (14.7-16.2)	15.3 (14.7-15.6)	15.8 (15.2-16.2)	NORMAL COLON
12	50	FEMALE	15.6 (15.2-16.4)	15.3 (14.7-15.5)	15.7 (15.1-16.4)	15.3 (14.7-16.1)	NORMAL COLON
13	80	MALE	15.6 (15.1-16.3)	15.6 (14.6-15.9)	15.6 (15.0-16.4)	15.8 (15.1-16.6)	DIVERTICULOSIS
14	38	MALE	16.0 (14.9-16.3)	15.4 (15.0-15.9)	16.3 (16.0-16.9)	14.9 (14.1-15.7)	NORMAL COLON
15	54	MALE	15.4 (14.7-15.8)	15.6 (15.1-16.3)	15.6 (15.1-15.9)	15.8 (15.0-16.3)	NORMAL COLON
16	65	FEMALE	14.6 (14.0-14.9)	15.9 (15.1-16.2)	13.9 (13.5-14.4)	15.9 (15.4-16.1)	NORMAL COLON
17	69	MALE	14.9 (14.4-15.3)	15.3 (15.0-15.9)	15.2 (14.7-15.5)	15.3 (15.0-16.2)	NORMAL COLON
18	65	MALE	15.6 (15.1-15.8)	15.6 (14.8-15.8)	16.0 (15.3-16.3)	15.3 (15.0-16.0)	NORMAL COLON
19	53	FEMALE	16.3 (15.7-16.5)	15.4 (15.0-15.9)	16.0 (15.7-16.4)	16.7 (15.8-16.9)	NORMAL COLON
20	71	MALE	15.3 (14.7-15.6)	15.8 (15.3-16.2)	15.3 (14.8-15.9)	15.4 (15.0-16.1)	DIVERTICULOSIS
21	43	FEMALE	14.9 (14.5-15.3)	15.9 (15.2-16.4)	15.6 (15.2-16.2)	15.0 (14.7-15.3)	NORMAL COLON
22	67	MALE	16.0 (15.6-16.3)	15.7 (15.5-16.1)	15.9 (15.5-16.3)	15.9 (15.3-16.3)	DIVERTICULOSIS
23	70	FEMALE	16.3 (16.0-16.8)	15.6 (15.2-15.9)	16.0 (15.3-16.2)	16.0 (15.4-16.3)	NORMAL COLON
24	63	MALE	16.3 (15.7-16.7)	15.7 (15.2-16.0)	15.9 (15.1-16.3)	16.0 (15.3-16.4)	NORMAL COLON
25	57	MALE	15.7 (15.2-15.9)	15.8 (15.3-16.1)	15.6 (15.0-16.0)	15.3 (15.1-15.9)	NORMAL COLON
average variability			4.8% 2.5-7.9	4.5% 2.3-6.9	4.8% 2.6-7.9	4.3% 2.2-8.0	

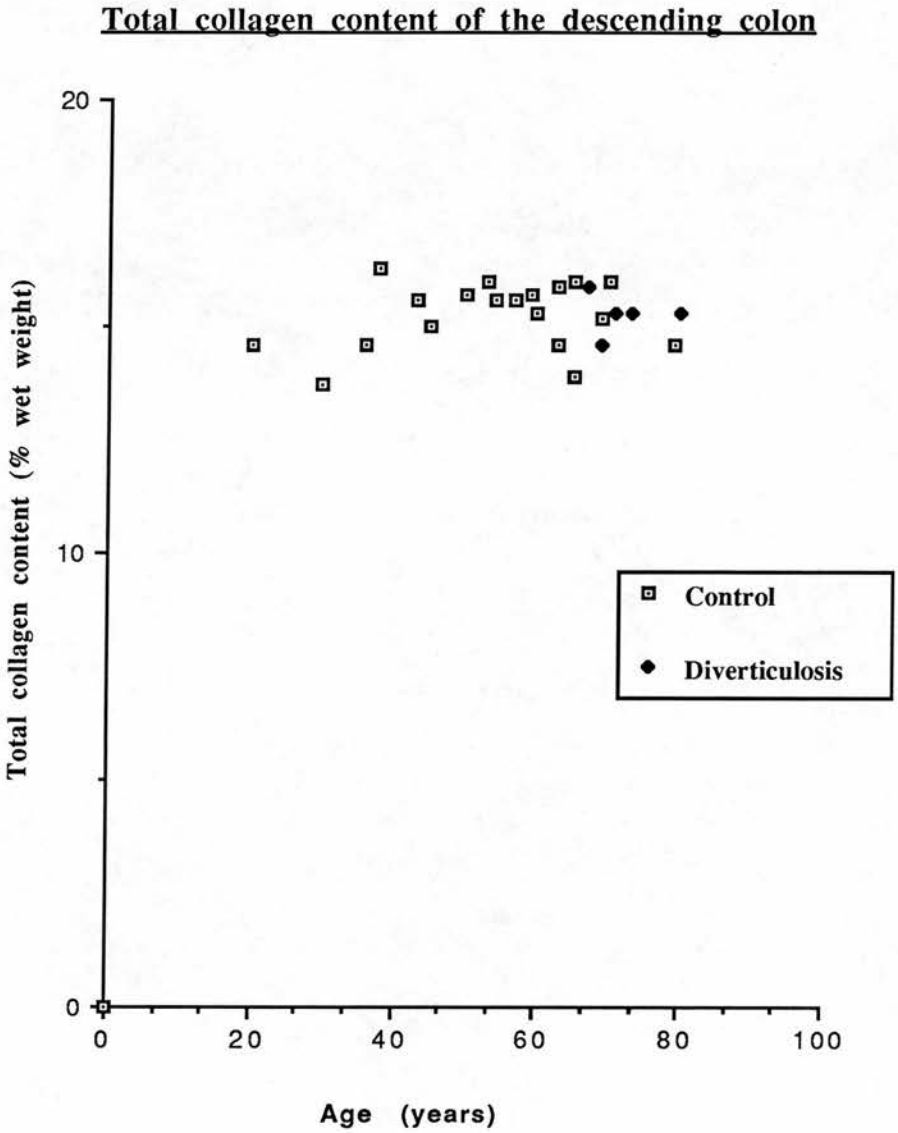
Table 4.1 : This table illustrates results of total collagen content of the four regions of the human colon. Results are expressed as a percentage of wet weight colon wall. Results were obtained in triplicate and are expressed as median and range of the three results. The variation of the results is shown as a percentage.



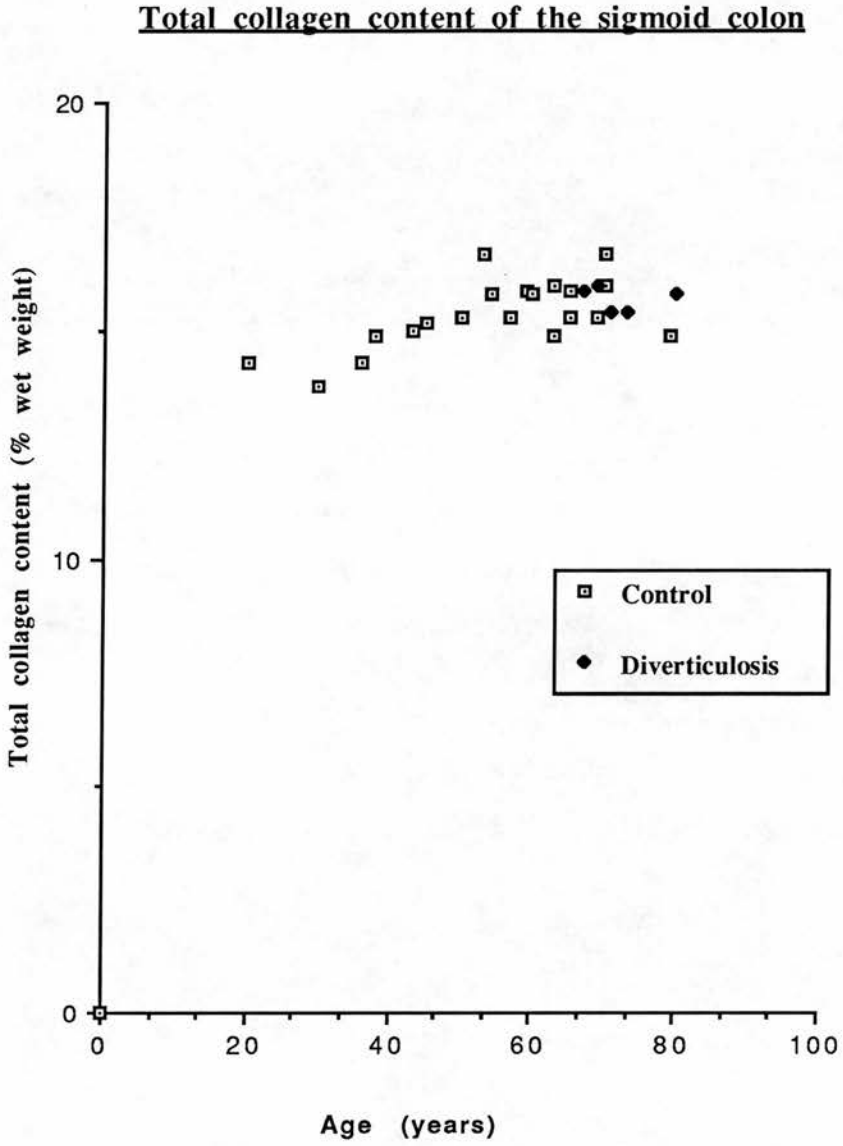
Graph 4.1: This graph illustrates the results for the measurement of the total collagen content of the ascending region of the human colon. (□) represents normal, healthy colons and (◆) those affected by colonic diverticulosis. The results are expressed as a percentage of the wet weight of the colon wall and are plotted against age of the subject in years.



Graph 4.2: This graph illustrates the results for the measurement of the total collagen content of the transverse region of the human colon. (□) represents normal, healthy colons and (◆) those affected by colonic diverticulosis. The results are expressed as a percentage of the wet weight of the colon wall and are plotted against the age of the subjects in years.



Graph 4.3: This graph illustrates the results for the measurement of the total collagen content of the descending region of the human colon. (□) represents normal, healthy colons and (◆) those affected by colonic diverticulosis. The results are expressed as a percentage of the wet weight of the colon wall and are plotted against the age of the subjects in years.



Graph 4.4: This graph illustrates the results for the measurement of the total collagen content of the sigmoid region of the human colon. (□) represents normal, healthy colons and (◆) those affected by colonic diverticulosis. The results are expressed as a percentage of the wet weight of the colon wall and are plotted against the age of the subjects in years.

SAMPLE	MEDIAN TOTAL COLL	MEAN TOTAL COLL	RANGE TOTAL COLL	p value
Overall subjects with normal colons age range 20-79				
ASCENDING	14.5	15.1	13.6-16.3	N.S.
TRANSVERSE	14.3	15.3	13.4-16.5	N.S.
DESCENDING	14.7	15.2	13.2-16.3	N.S.
SIGMOID	14.6	15.4	13.4-16.7	N.S.
Age matched subjects with normal colons age range 67-79				
ASCENDING	14.9	15.4	14.3-16.3	N.S.
TRANSVERSE	14.9	15.6	14.3-16.0	N.S.
DESCENDING	14.7	15.8	14.5-16.0	N.S.
SIGMOID	15.3	15.5	14.9-16.7	N.S.
Subjects with colonic diverticulosis				
ASCENDING	15.3	15.8	15.8-16.3	N.S.
TRANSVERSE	15.4	15.9	14.9-15.3	N.S.
DESCENDING	15.6	15.9	15.3-15.9	N.S.
SIGMOID	15.6	15.9	15.4-16.3	N.S.

Sample	Mean	Standard deviation	Standard error	p value	Age range
Ascending total collagen against age.	13.80	0.40	0.9	non significant	20-80
Transverse total collagen against age.	13.63	0.7	1.12	non significant	20-80
Descending total collagen against age.	14.0	0.8	1.01	non significant	20-80
Sigmoid total collagen against age.	13.86	0.32	1.01	non significant	20-80

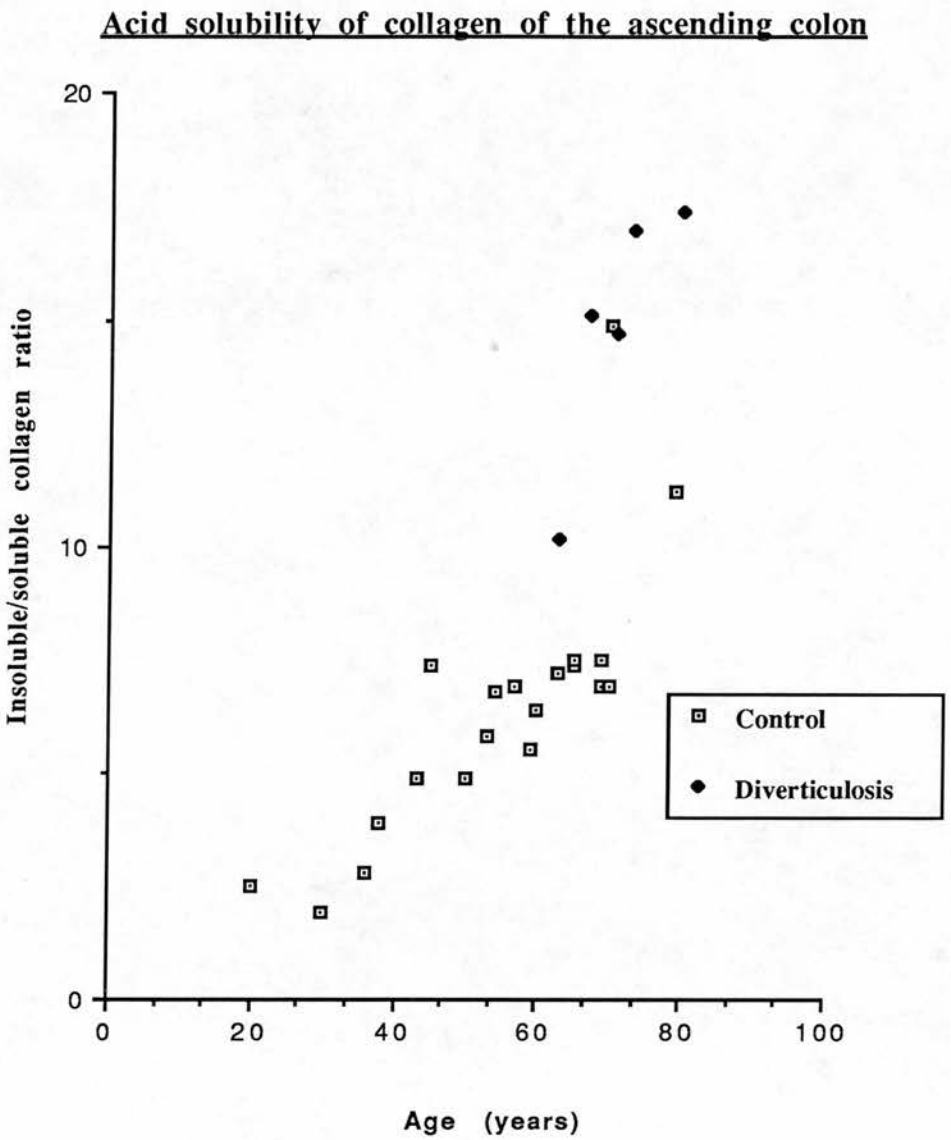
Table 4.2: This table illustrates statistical analysis of the results for the measurement of total collagen of the human colon. The median and range of the results are shown. Each section of the colon is compared with the sigmoid region and the age of the subjects. N.S. represents a non significant result.

4.1.2 Measurement of the acid solubility of human colon collagen.

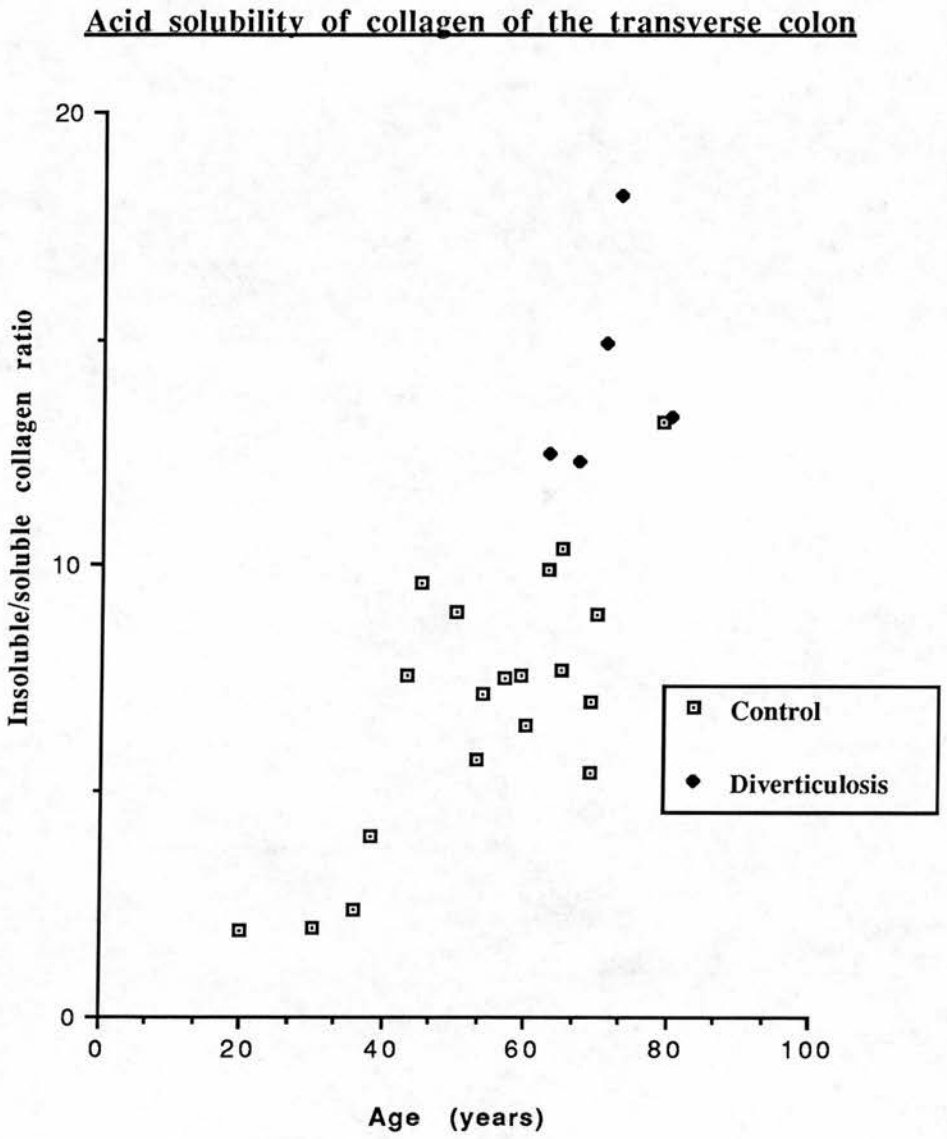
The acid solubility of the collagen from the human bowel was measured in all four regions of the colon, and in all twenty five sections collected, using the methodology described in Chapter three, section 3.2. The results were obtained in triplicate and are expressed as median and range of the three results. The results are shown in Table 4.3 and are illustrated graphically in Graphs 4.5-4.8. Analysis of these results is shown on Table 4.4.

PATIENT	AGE	SEX	ASC.	TRANS	DESC.	SIGMOID	DIVERTICULOSIS ?
1	20	MALE	2.5 (1.9-2.9)	1.9 (1.7-2.3)	2.3 (2.0-2.6)	2.5 (2.2-2.7)	NORMAL COLON
2	30	FEMALE	1.9 (1.5-2.6)	2.0 (1.7-2.3)	1.7 (1.5-2.1)	1.9 (1.7-2.1)	NORMAL COLON
3	36	FEMALE	2.8 (2.2-3.2)	2.4 (2.1-2.9)	3.5 (3.1-3.7)	2.8 (2.6-3.0)	NORMAL COLON
4	45	FEMALE	7.4 (6.1-7.8)	9.6 (9.3-10.4)	8.4 (8.0-8.9)	7.4 (7.1-7.7)	NORMAL COLON
5	59	MALE	5.5 (5.0-6.1)	7.6 (7.1-7.9)	6.0 (5.8-6.3)	5.5 (5.3-5.7)	NORMAL COLON
6	63	FEMALE	10.2 (9.4-10.9)	12.5 (12.0-13.2)	10.3 (9.9-10.6)	14.9 (14.6-15.1)	DIVERTICULOSIS
7	69	FEMALE	7.5 (7.1-8.4)	5.4 (4.9-5.8)	8.8 (8.6-9.2)	5.1 (4.9-5.3)	NORMAL COLON
8	70	MALE	14.9 (13.7-15.4)	8.9 (8.3-9.4)	13.3 (13.0-13.7)	11.6 (11.3-11.9)	NORMAL COLON
9	73	FEMALE	17.0 (16.6-17.9)	18.2 (17.7-18.9)	19.8 (19.6-20.3)	17.0 (16.7-17.3)	DIVERTICULOSIS
10	79	FEMALE	11.2 (10.6-11.7)	13.2 (12.7-13.8)	8.9 (8.6-9.1)	15.4 (15.1-15.7)	NORMAL COLON
11	60	MALE	6.4 (5.8-6.9)	6.5 (6.0-7.1)	6.9 (6.8-7.1)	6.4 (6.1-6.8)	NORMAL COLON
12	50	FEMALE	4.9 (4.4-5.2)	9.0 (8.4-9.5)	8.8 (8.6-9.0)	4.9 (4.6-5.2)	NORMAL COLON
13	80	MALE	17.4 (16.6-18.1)	13.3 (12.8-13.8)	19.9 (19.6-20.1)	23.8 (23.5-24.2)	DIVERTICULOSIS
14	38	MALE	3.9 (3.6-4.2)	4.0 (3.3-4.4)	3.7 (3.3-3.9)	3.9 (3.6-4.1)	NORMAL COLON
15	54	MALE	6.8 (6.0-7.5)	7.2 (6.6-7.7)	8.0 (7.6-8.3)	6.8 (6.6-7.0)	NORMAL COLON
16	65	FEMALE	7.4 (7.1-8.0)	7.7 (7.2-8.3)	10.7 (10.3-11.0)	7.4 (7.1-7.7)	NORMAL COLON
17	69	MALE	6.9 (6.2-7.3)	7.0 (6.5-7.6)	7.0 (6.6-7.2)	6.9 (6.6-7.1)	NORMAL COLON
18	65	MALE	7.5 (7.0-8.3)	10.4 (9.9-10.9)	9.9 (9.6-10.1)	7.5 (7.2-7.8)	NORMAL COLON
19	53	FEMALE	5.8 (5.1-6.3)	5.7 (5.1-6.4)	6.3 (6.0-6.6)	5.5 (5.3-5.8)	NORMAL COLON
20	71	MALE	14.7 (14.1-15.5)	14.9 (14.6-15.5)	12.0 (11.7-12.3)	14.7 (14.5-14.9)	DIVERTICULOSIS
21	43	FEMALE	4.9 (4.2-5.4)	7.6 (7.1-8.4)	8.4 (8.1-8.6)	5.5 (5.3-5.8)	NORMAL COLON
22	67	MALE	15.1 (14.6-15.8)	12.3 (11.8-12.9)	13.0 (12.7-13.2)	15.1 (14.8-15.3)	DIVERTICULOSIS
23	70	FEMALE	6.9 (6.1-7.4)	8.9 (8.2-9.3)	9.2 (8.9-9.5)	8.9 (8.7-9.2)	NORMAL COLON
24	63	MALE	7.2 (6.7-7.7)	9.9 (9.1-10.4)	9.0 (8.7-9.3)	7.1 (6.8-7.3)	NORMAL COLON
25	57	MALE	6.9 (6.2-7.5)	7.5 (6.9-7.9)	6.0 (5.8-6.1)	5.8 (5.6-6.1)	NORMAL COLON
average variability			7.9% 5.0-10.9	7.5% 4.6-11.2	7.9% 4.3-11.4	8.1% 4.2-11.9	

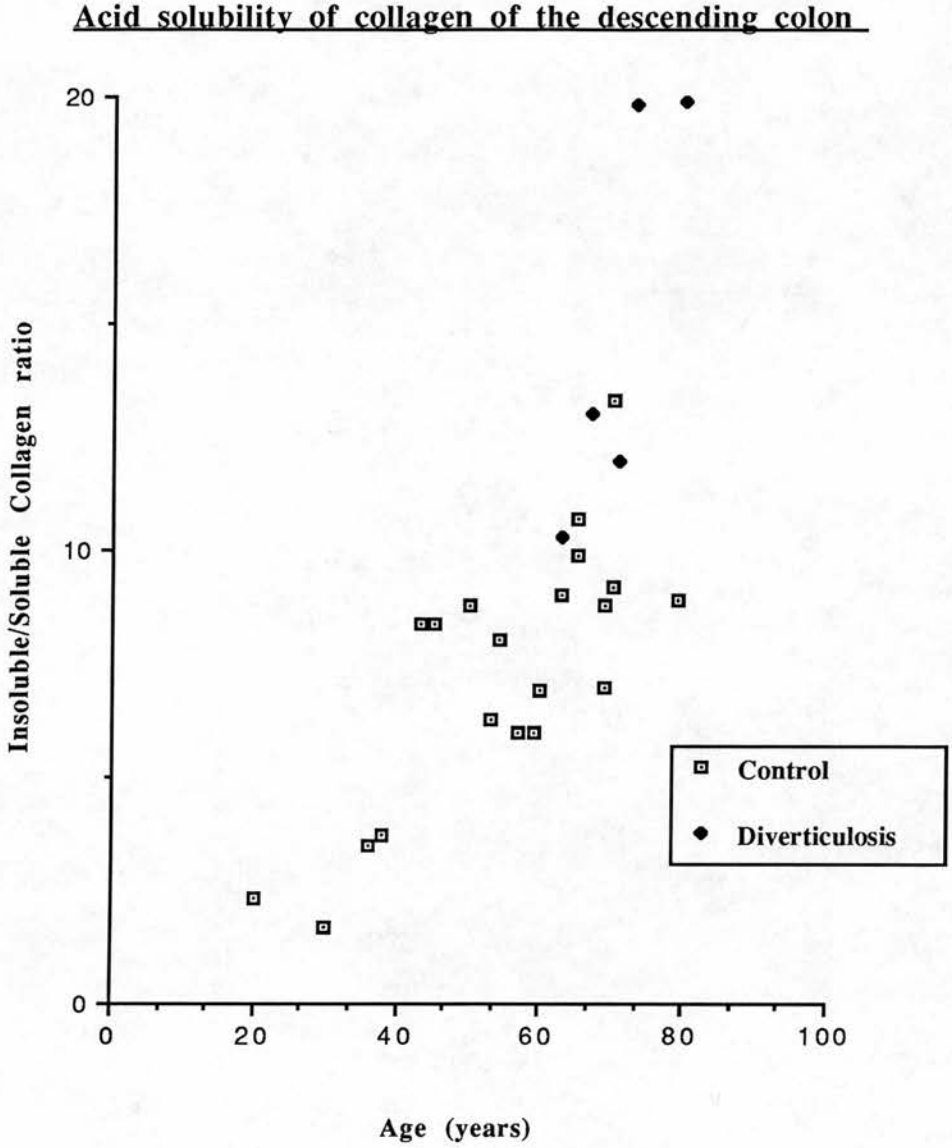
Table 4.3: This table illustrates the results for the measurement of the acid solubility of the collagen from all four regions of the colon. The results are expressed as insoluble : soluble collagen ratio as median and range of the three results. The variation of the results is shown as a percentage.



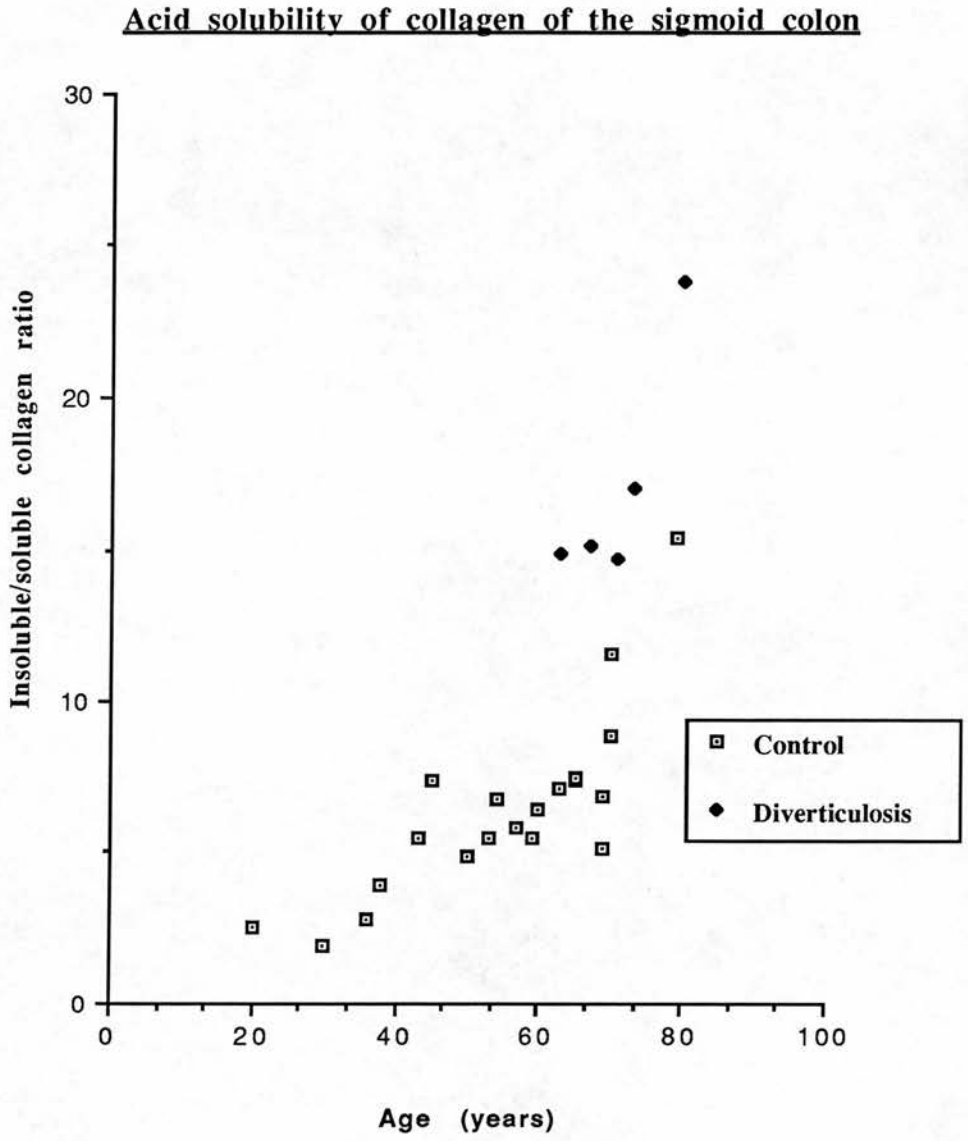
Graph 4.5: This graph illustrates the results for the measurement of the acid solubility of collagen from the human ascending colon. (□) represents normal, healthy colons and (◆) those affected by colonic diverticulosis. Results are expressed as a ratio of insoluble : soluble collagen as found in the colon wall, and are plotted against the age of the subject in years.



Graph 4.6: This graph illustrates the results for the measurement of the acid solubility of collagen from the human transverse colon. (□) represents normal, healthy colons and (◆) those affected by colonic diverticulosis. Results are expressed as a ratio of insoluble : soluble collagen as found in the colon wall, and are plotted against the age of the subject in years.



Graph 4.7: This graph illustrates the results for the measurement of the acid solubility of collagen from the human descending colon. (⊡) represents normal, healthy colons and (◆) those affected by colonic diverticulosis. Results are expressed as a ratio of insoluble : soluble collagen as found in the colon wall, and are plotted against the age of the subject in years.



Graph 4.8: This graph illustrates the results for the measurement of the acid solubility of collagen from the human sigmoid colon. (□) represents normal, healthy colons and (◆) those affected by colonic diverticulosis. Results are expressed as a ratio of insoluble : soluble collagen as found in the colon wall, and are plotted against the age of the subject in years.

SAMPLE	MEDIAN SOL INDEX	MEAN SOL INDEX	RANGE SOL INDEX	p value
Overall subjects with normal colons age range 20-79				
ASCENDING	6.0	6.30	1.9-14.9	N.S.
TRANSVERSE	7.4	7.10	1.9-13.2	N.S.
DESCENDING	7.6	7.34	1.7-13.3	N.S.
SIGMOID	6.1	7.98	1.9-15.4	N.S.
Age matched subjects with normal colons age range 67-79				
ASCENDING	11.1	8.28	6.9-14.9	<0.001
TRANSVERSE	11.8	8.68	5.4-13.2	<0.001
DESCENDING	11.9	9.44	7.0-13.3	<0.001
SIGMOID	11.3	9.98	5.1-15.4	<0.001
Subjects with colonic diverticulosis				
ASCENDING	14.4	13.44	10.2-17.4	<0.05
TRANSVERSE	13.2	14.88	10.5-18.2	<0.05
DESCENDING	12.0	15.00	10.3-19.9	<0.05
SIGMOID	14.7	17.1	14.9-23.8	<0.001

Sample	Mean	Standard deviation	Standard error	p value	Age range
Ascending solubility ratio against age.	17.9	1.0	0.19	p<0.001	20-80
Transverse solubility ratio against age.	19.4	1.2	0.12	p<0.01	20-80
Descending solubility ratio against age.	21.7	1.4	0.31	p<0.001	20-80
Sigmoid solubility ratio against age.	22.8	1.2	0.28	p<0.01	20-80

Table 4.4 : This table illustrates the analysis of the results obtained for the measurement of the acid solubility of the collagen of the colon. This table illustrates the median and range of the results and the p values. The results are compared with the sigmoid colon and the age of the subject. N.S. represents a non significant result.

4.1.3 X-ray fibre diffraction.

X-ray fibre diffraction, and subsequent data analysis is a complex and laborious technique. Therefore, all of the samples collected could not be examined by X-ray fibre diffraction during this thesis. It was decided that samples from the following categories should be analysed in order to determine any observable differences in collagen structure or packing between healthy and pathological samples.

Category 1 Healthy, young samples- all four regions of the colon.

Category 2 Healthy, aged samples - all four regions of the colon.

Category 3 Diseased, aged, samples- all four regions of the colon.

The resulting photographs produced from these analyses are shown in Plates 4.1-4.8.

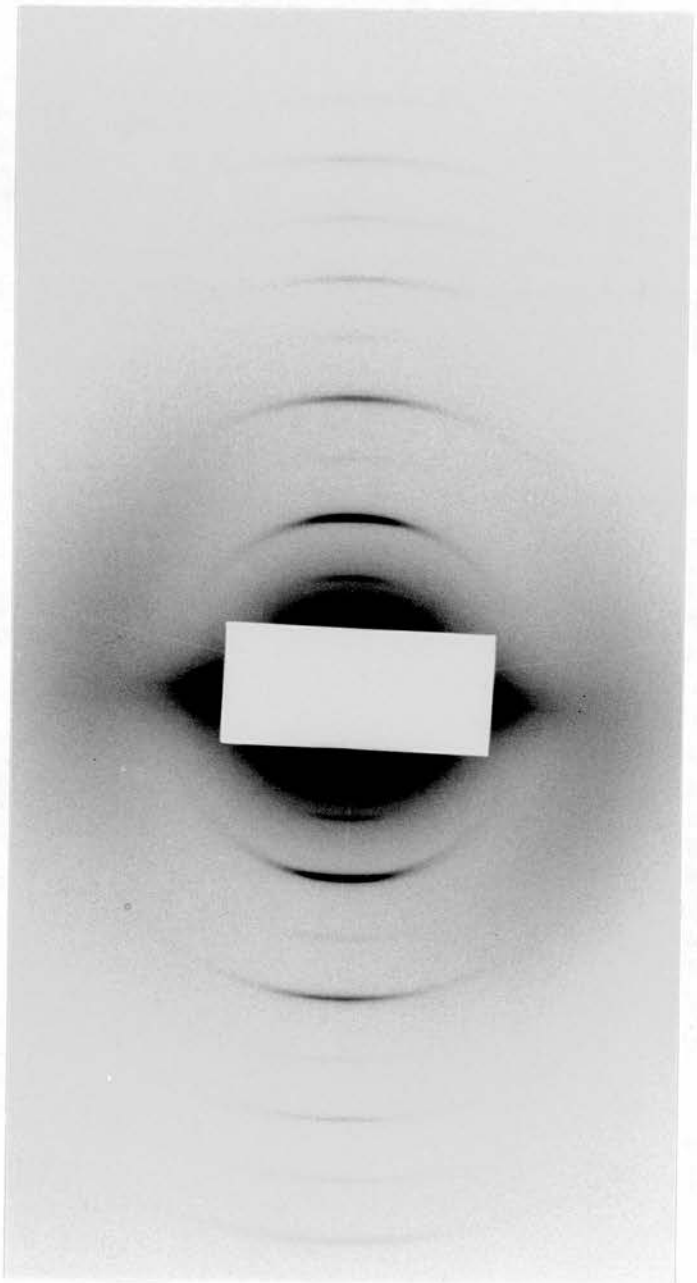


Plate 4.1: This photograph illustrates a small angle X-ray diffraction pattern of a healthy, young sample. This pattern was recorded on a 3m camera on station 8.2, S.R.S., Daresbury, England, U.K..

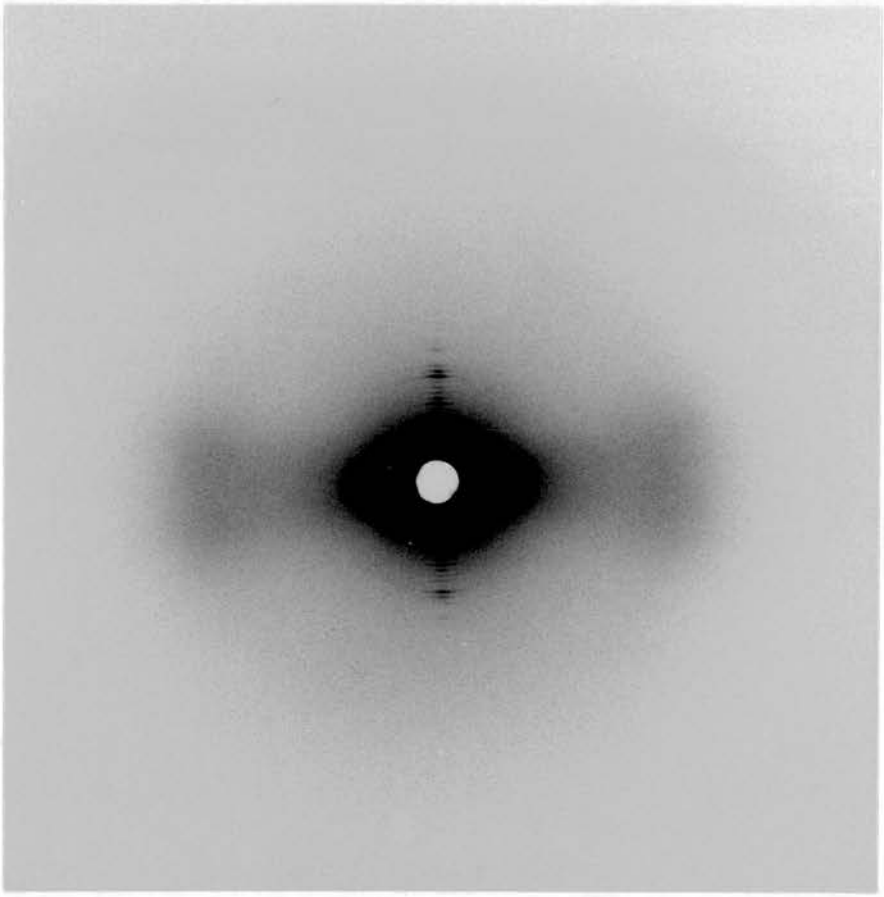


Plate 4.2: This photograph illustrates a high angle X-ray diffraction pattern of a healthy, young sample. This pattern was recorded using a 0.3m camera on station 7.2, S.R.S., Daresbury, England, U.K..

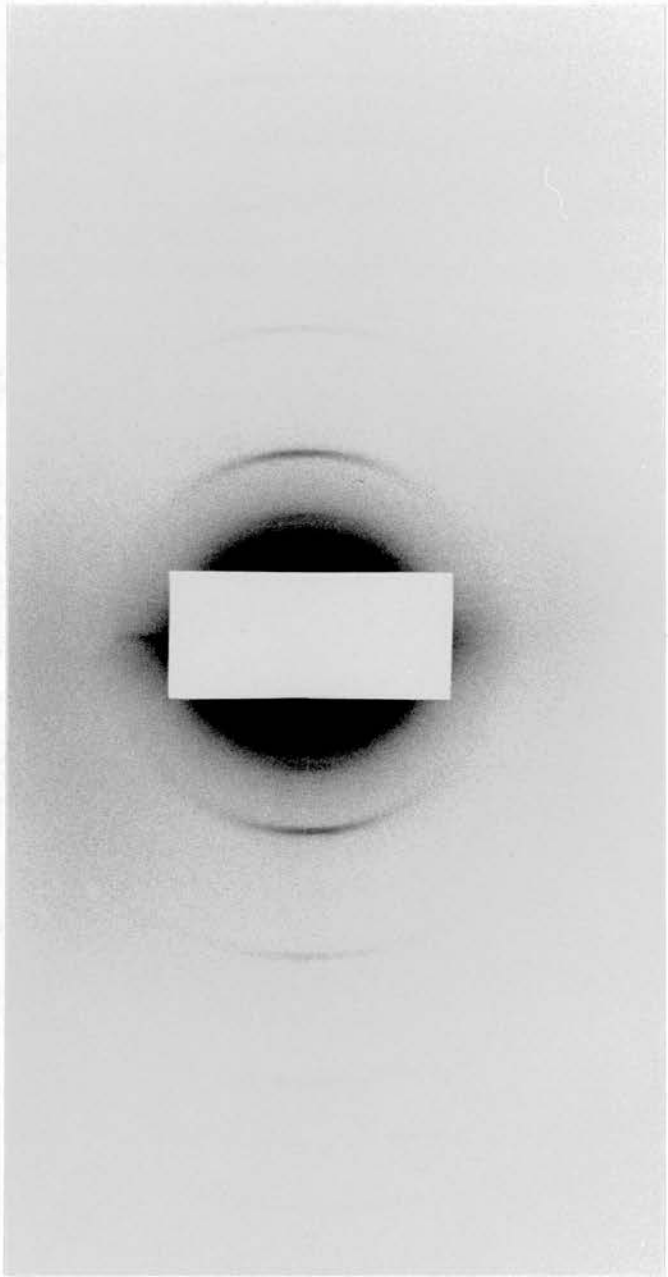


Plate 4.3 : This photograph illustrates a small angle X-ray diffraction pattern of an healthy, aged sample. This pattern was recorded using a 3m camera on station 8.2 at S.R.S., Daresbury, England, U.K..

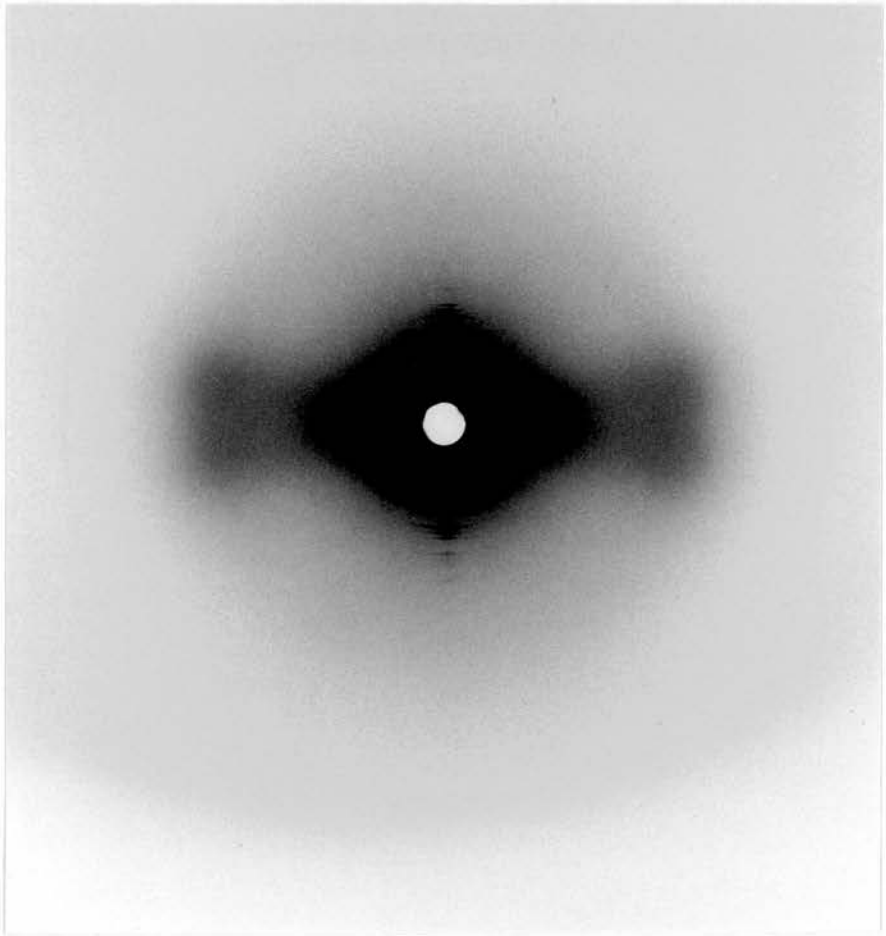


Plate 4.4 : This photograph illustrates a high angle X-ray diffraction pattern of a healthy, aged sample. This pattern was recorded using a 0.3m camera on station 7.2, S.R.S., Daresbury, England, U.K..

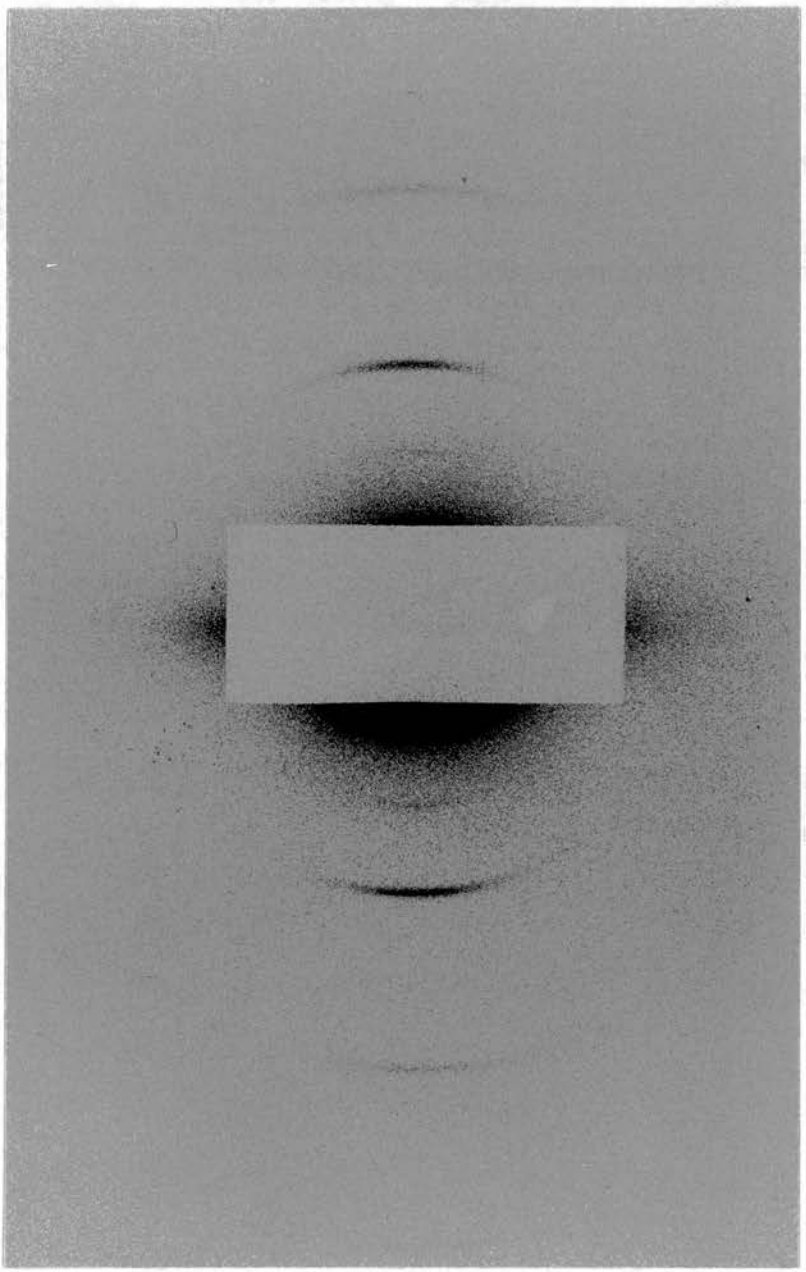


Plate 4.5: This photograph illustrates a small angle X-ray diffraction pattern of a diseased, aged sample. This pattern was recorded using a 3m camera on station 8.2, S.R.S., Daresbury, England, U.K..

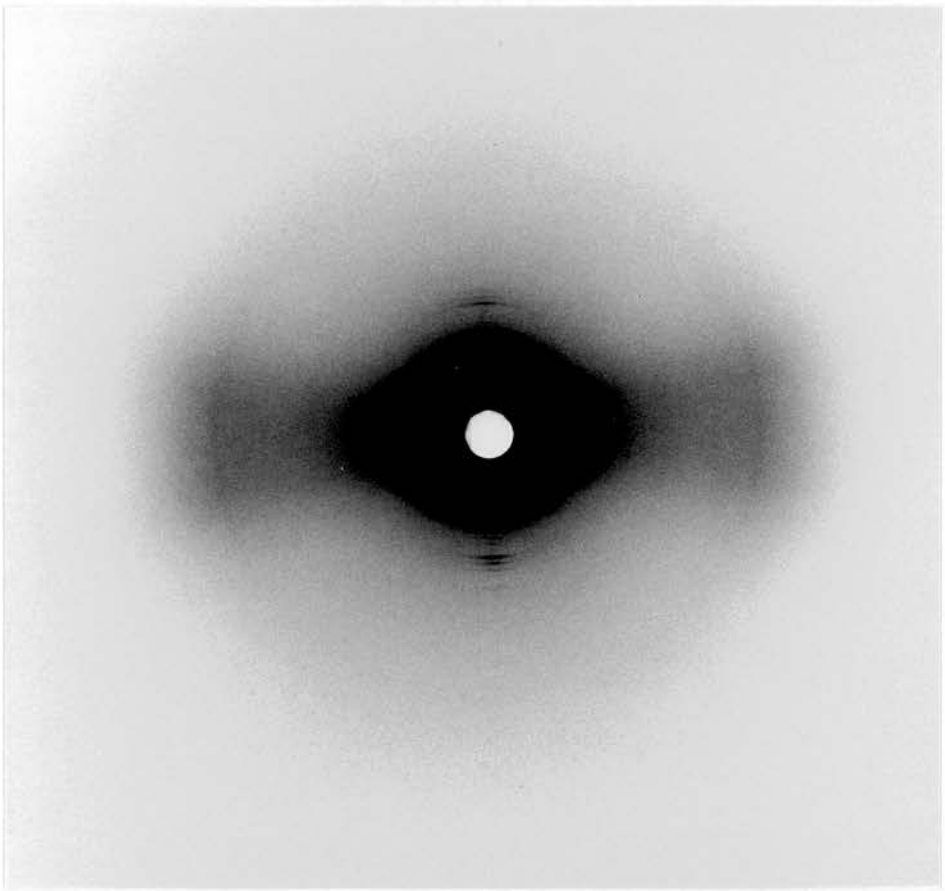


Plate 4.6 : This photograph illustrates a high angle X-ray diffraction pattern of a diseased, aged sample. This pattern was recorded using a 0.3m camera on station 7.2, S.R.S., Daresbury, England, U.K..

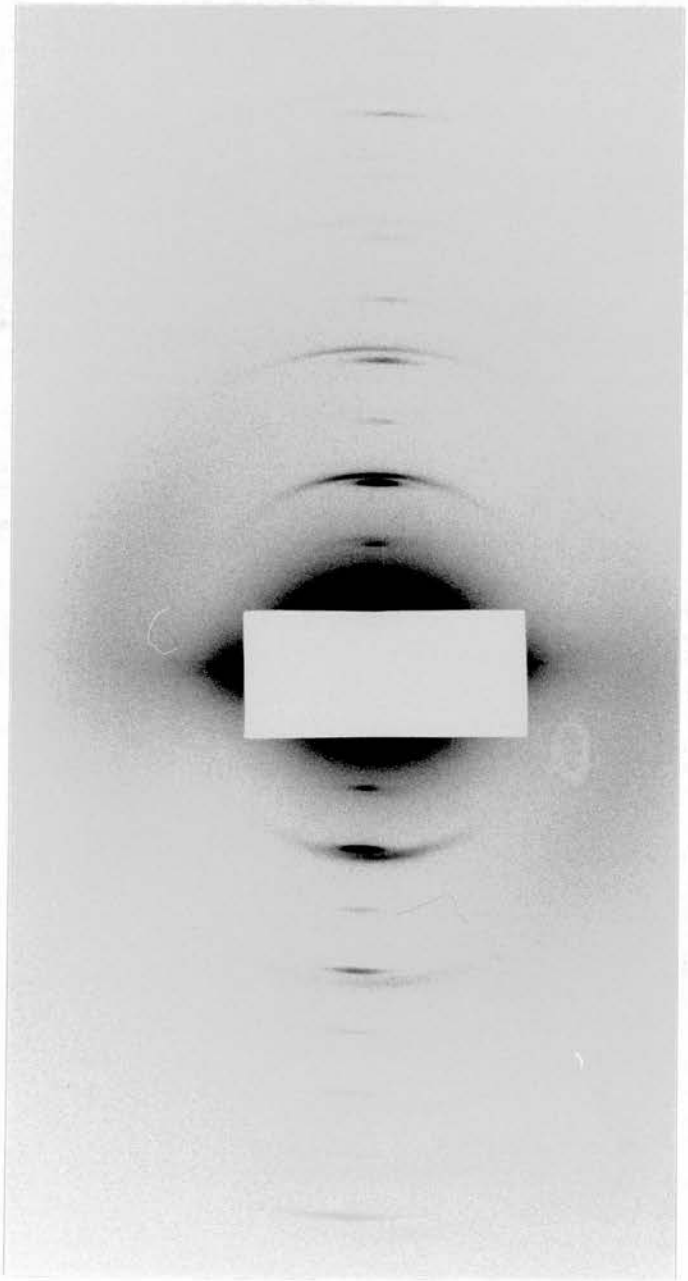


Plate 4.7: This photograph illustrates a small angle X-ray diffraction pattern of human colon collagen with rat tail tendon collagen. The difference in the D period is illustrated. This pattern was recorded using a 1m camera on station 8.2, S.R.S., Daresbury, England, U.K..

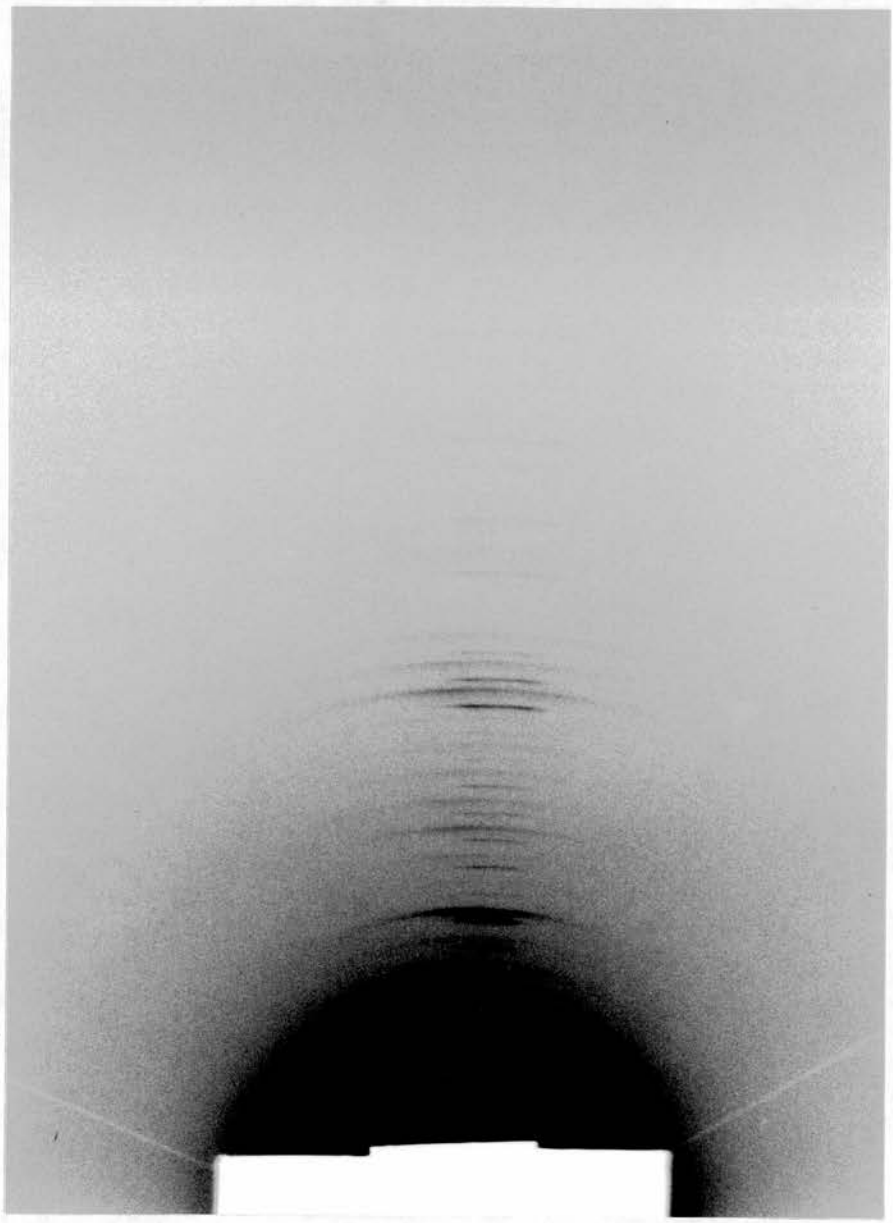


Plate 4.8: This photograph illustrates a high angle diffraction pattern of human colon collagen with rat tail tendon collagen, illustrating the difference in D period. This pattern was recorded using a 0.3m camera on station 7.2, S.R.S., Daresbury, England, U.K..

4.2 Analysis of X-ray fibre diffraction patterns.

All of the diffraction patterns illustrated in this thesis were analysed using the same method. A preliminary study of fibre diffraction pattern quality was made on site at the S.R.S., Daresbury, U.K.. This was to determine if the sample had been correctly aligned and that the direct beam of undiffracted X-rays had been collected by the backstop. Since the synchrotron produces a variable amount of flux depending on its energy status, the amount of exposure given to the sample was judged by the density of diffraction peaks on the film. The exposure was repeated, and the exposure time increased, if the sample did not produce a discernable diffraction pattern. No beam damage was observed, or expected, in the timescales used for these experiments.

The diffraction patterns considered here can be regarded as consisting of two main series of reflections. These are the meridional and equatorial reflections. Meridional reflections correspond to the variation in electron density along the axis of the collagen fibres. The ordering of collagen in fibrils in this direction is indicated by the discrete peaks of diffraction. This can be contrasted with the equatorial reflections. Equatorial reflections correspond to the variation in electron density due to the packing of the collagen molecules in a fibre. The relatively non specific packing of the collagen molecules in a lateral plane is indicated by the lack of discrete reflections in this direction. In all the difffraction patterns shown, the meridional reflections are in the vertical plane, whilst the equatorial reflections are in the horizontal plane. Low angle diffraction patterns (obtained with a camera of 1m or more) do not contain the equatorial region since observable diffraction in this region only occurs at relatively high angles.

The first analysis of fibre diffraction patterns was to make a visual examination of the equatorial reflections. This was in order to observe if the equatorial

region consisted of one or two discernable regions of diffuse scatter. If the equatorial reflections were observed as one peak centred on the equator, the packing of the molecules in the collagen fibril could be regarded as being relatively straightforward. The diffraction peak in this case would be due to the consistent packing of collagen molecules in a hexagonally close packed array. The diffuse nature of the equatorial peak demonstrated that there was a spread in the distances between molecules. This could be attributed to the inherent disorder of packing in collagen fibrils. The existence of two lobes of intensity, one above and one below the equator, implies that the collagen fibrils contain some degree of supercoiling. The films were scanned to measure the position and intensity of the meridional reflections, using a Joyce Loebl Chromoscan 3 densitometer. This allowed analysis of the relative intensities of the meridional diffraction peaks. For each sample a pack of five films was used. This allowed the relatively weak diffraction intensities to be recorded on the first few films. Peaks that had the highest intensity would saturate the first film. The films lower down the pack were used to record the unsaturated intensity of these peaks. When five films were used, the intensity of the first thirty meridional reflections could be determined. The intensity of unsaturated diffraction peaks from each film was used in order to obtain a complete set of unsaturated intensities. The intensity of the first meridional order for each sample was scaled to a value of 10000. The intensities of all other meridional reflections for each sample were scaled accordingly. In each case the error of measurement is proportional to the square root of the intensity recorded. This is standard for fibre diffraction. The resultant intensities are shown on Tables 4.5-4.8.

Order	Young, healthy	Aged, healthy	Aged, diseased.
1	10000	10000	10000
2	152	132	102
3	1428	1352	1298
4	143	124	111
5	601	552	513
6	213	199	181
7	204	199	178
8	116	108	99
9	592	581	577
10	135	108	93
11	62	53	49
12	214	209	199
13	28	23	19
14	59	54	50
15	43	38	31
16	36	29	23
17	65	33	28
18	11	8	5
19	28	25	21
20	179	167	156
21	169	154	139
22	73	69	58
23	5	3	2
24	2	1	1
25	113	104	99
26	29	22	20
27	72	69	61
28	2	2	2
29	33	30	26
30	86	82	80

Table 4.5 : This table illustrates a list of intensities for the first 30 orders of diffraction of human colon collagen from the ascending region. All values are scaled to a first order of 10000. The error of intensity measurement for each reflection is in the range 5-10%.

Order	Young, healthy	Aged, healthy	Aged, diseased.
1	10000	10000	100000
2	153	150	139
3	1418	1394	1362
4	139	128	118
5	603	600	590
6	208	200	189
7	201	196	180
8	118	104	98
9	601	588	571
10	138	127	120
11	61	53	49
12	211	199	181
13	27	23	19
14	60	54	46
15	42	37	31
16	37	34	27
17	63	60	52
18	12	8	6
19	27	23	18
20	178	159	145
21	163	151	140
22	71	67	57
23	5	5	3
24	2	1	1
25	112	108	92
26	27	24	20
27	74	69	60
28	2	2	2
29	33	28	22
30	88	75	67

Table 4.6 : This table illustrates a list of intensities for the first 30 orders of diffraction of human colon collagen from the transverse region. All values are scaled to a first order of 10000. The error of intensity measurement for each reflection is in the range 5-10%.

Order	Young, healthy	Aged, healthy	Aged, diseased.
1	10000	10000	10000
2	158	147	137
3	1432	1419	1384
4	142	135	123
5	600	590	571
6	206	200	191
7	200	191	180
8	117	111	103
9	600	591	563
10	135	128	119
11	58	55	48
12	207	201	190
13	23	20	16
14	58	51	42
15	40	36	28
16	36	31	25
17	60	55	49
18	11	10	8
19	28	25	19
20	176	163	151
21	162	149	132
22	70	64	51
23	5	4	3
24	2	2	1
25	111	106	99
26	26	23	18
27	72	68	52
28	2	2	2
29	30	27	23
30	85	81	71

Table 4.7 : This table illustrates a list of intensities for the first 30 orders of diffraction of human colon collagen from the descending region. All values are scaled to a first order of 10000. The error of intensity measurement for each reflection is in the range 5-10%.

Order	Young, healthy	Aged, healthy	Aged, diseased.
1	10000	10000	10000
2	159	149	132
3	1438	1401	1381
4	150	147	130
5	603	595	580
6	204	195	177
7	200	191	182
8	118	105	88
9	599	581	567
10	133	127	115
11	54	48	40
12	201	190	183
13	24	20	16
14	59	51	44
15	42	35	30
16	34	28	21
17	62	51	46
18	12	8	5
19	27	21	16
20	170	158	141
21	160	148	130
22	73	66	60
23	5	3	2
24	2	2	1
25	114	109	101
26	25	20	16
27	73	66	59
28	2	2	2
29	33	30	24
30	87	84	77

Table 4.8 : This table illustrates a list of intensities for the first 30 orders of diffraction of human colon collagen from the sigmoid region. All values are scaled to a first order of 10000. The error of intensity measurement for each reflection is in the range of 5-10%.

4.2.1 Analysis of recorded intensities.

The diffraction data illustrate that the general trend of decreasing intensity with respect to order, is greater in the diseased samples than in the healthy ones. There are also significant differences between the healthy, young and healthy, aged data (e.g. order 5, $p<0.001$). The differences can be attributed to the degree of organisation of the collagen within a fibril. As the order number increases, the electron density function that produces each reflection corresponds to a finer detail. The data presented therefore indicates that the low level resolution of collagen in the fibrils is generally similar between samples. The changes due to disordering of structures are obviously amplified at higher resolutions. In diseased tissue, the relative axial ordering of collagen molecules in a fibril is less than in the healthy tissues. This implies an increased disorder in the diseased samples. It is possible that the disorder has a contribution from the increase in crosslinkages between collagen molecules. These may cause randomised deviations in the packing of the molecules. This results in the intensity of the meridional reflections being spread out over a large arc in reciprocal space. The axial crystalline nature of the fibril is lost, and diffraction intensity cannot be distinguished from film noise.

The differences which have been detected between the healthy, young and healthy, aged samples could be related to the decrease in acid solubility which has been determined associated with an increase in age. A decrease in acid solubility could be explained by increased glycation related crosslinking. Since this process is non enzymatic, it may be relatively non specific, and therefore may induce disorder in fibril structure.

4.2.2 The equatorial reflections.

The equatorial reflections provide information on the lateral packing of the collagen molecules. All of the diffraction data which have been obtained

exhibited two weak lobes of intensity, one above and one below the equator. This indicates that the collagen molecules are supercoiled and are coiled around the fibril axis. This produces an apparent tilt of the molecules relative to the fibril axis of 9^0. The presence of supercoiling of collagen fibrils has also been noted by Christmann *et al*, in sheep skin (Christmann D. *et al*, 1989). This feature is thought to occur commonly in tisues that contain type I and type III collagen and has been reviewed (Eikenberry E.F. *et al,* 1984).

4.2.3 D-periodicity.

From the diffraction patterns obtained, it is evident that the D period of human submucosal collagen is shorter than that for type I collagen from rat tail tendon. Type I collagen has a D period of 67.0nm, and collagen from human colon has a D period of 65.5nm. This difference may be explained by the presence of supercoiling of the collagen molecules as exhibited by the equatorial splitting (Brodsky B., Eikenberry E.F. and Cassidy K., 1980). Therefore although the length of the axial repeating unit in these samples may be 67.0nm their projection with a 9^0 tilt relative to the fibre axis will produce an apparent length of 67.0 cos 9^0 i.e. 65.5nm.

Chapter 5

The effect of diet

on rat colonic

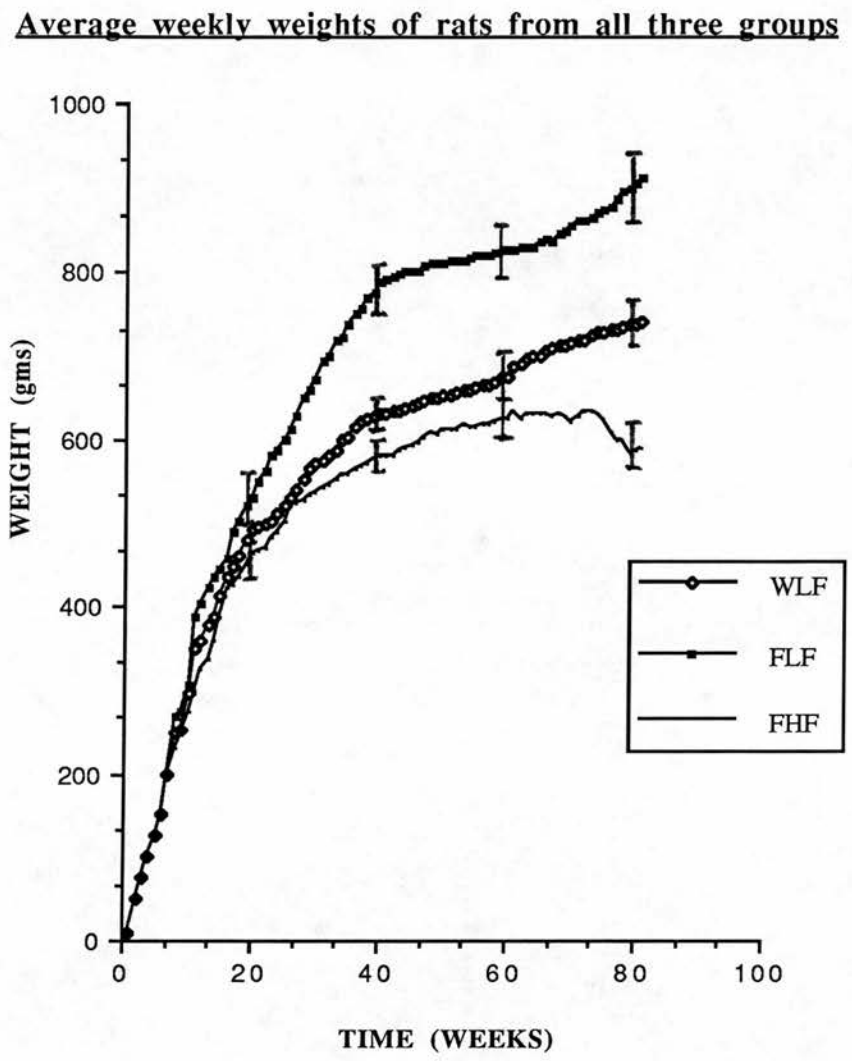
collagen.

5.1 Results of the study of the effect of diet on the rat colon wall. A model for human colonic diverticulosis.

This study was concerned with the analysis of various tissues from three groups of rats which were fed on different diets. The experiment aimed to determine the effect of diet on the structure of the collagen of the rat colon in particular relation to colonic diverticulosis. After *post-mortem*, all sections were treated using the methodology described in Chapter four. The use of two different diets allowed analysis of any dietary induced tissue differences.

5.1.1 Health of animals throughout the experiment.

The health of all of the rats was examined throughout the experiment. This included examining the teeth, skin, mobility and more importantly body weight. These health checks were carried out on a weekly basis. The results of the weight measurements are illustrated graphically on Graph 5.1. The animals fed on the low fibre diet are of a much higher weight than those fed on the high fibre diet (see Plate 5.1). Unfortunately, four of the rats died before the end of the experiment and are not included in the tissue analysis. All of the rats were known to die of causes which were not related to the diets which they were being fed. Three of the rats died of middle ear disease which is a common complaint in rodents.



Graph 5.1 : This graph illustrates the growth and weight gain of the animals from the three dietary groups. The average weight in grams is plotted against time. The three groups are shown as F.L.F., F.H.F. and W.L.F., which denotes familial low fibre fed rats, familial high fibre fed rats and weaned low fibre fed rats respectively.

Plate 5.1: This photograph shows the different weights of representative animals from the three groups, together with a young animal (3 months) for comparison. This photograph clearly illustrates the difference in weight due to the different diets. The rats are from left to right, familial low fibre fed, weaned low fibre fed, familial high fibre fed and a young animal (3 months) for comparison.

5.1.2 Results of faecal output collection.

As stated in Chapter three, a faecal collection study was undertaken over a twenty four hour period. Faeces were collected and food intake was measured over the same time period. The results are shown in Table 5.1. These results demonstrate that the two groups fed on the low fibre diet have a very low faecal output compared with the animals fed on the high fibre diet. The food intake values are similar for the three different groups.

5.1.3 *Post-mortem* examination.

All rats were sacrificed at approximately the same time and the required tissues removed immediately. The general condition of each animal was examined, together with more specific examination of the lungs, heart, pancreas, liver, kidneys and testes, for any abnormalities or tumours. The results of the *post-mortems* were tabulated and are shown in Tables 5.2 - 5.4. The position and number of abnormalities or tumours are shown in each table.

Experimental group 1 (Familial Low Fibre)

RAT NO.	BODY WEIGHT	FOOD INTAKE	FAECAL OUTPUT
1	578g	16.0g	1.15g
12	624g	16.4g	0.77g
13	620g	16.3g	1.37g
15	622g	16.4g	0.95g
20	628g	16.4g	2.16g
			$\bar{x}$ = 1.28g
			s.d. = 0.54

Experimental group 2 (Weaned Low Fibre)

RAT NO.	BODY WEIGHT	FOOD INTAKE	FAECAL OUTPUT
2	628g	15.6g	1.73g
4	626g	16.2g	1.00g
9	618g	16.0g	0.85g
13	632g	16.2g	1.36g
14	618g	16.1g	1.50g
			$\bar{x}$ =1.29g
			s.d. = 0.36

Control group (Familial High Fibre)

RAT NO.	BODY WEIGHT	FOOD INTAKE	FAECAL OUTPUT
10	632g	17.3g	5.64g
11	614g	17.2g	6.09g
12	612g	17.5g	6.06g
16	626g	17.3g	4.84g
18	612g	17.6g	4.11g
			$\bar{x}$ =5.35g
			s.d. = 0.86

Table 5.1 : This table illustrates the results for the faecal collection experiment. Results are shown as grams faeces produced in 24 hours, and as a mean of each of the groups of five rats. Food intake measurements are shown as grams taken in 24 hours. Results are expressed as mean +/- standard deviation (s.d.) of the mean.

P.M. REPORT SHEET

FAMILIAL HIGH FIBRE GROUP

CONTROL GROUP

***POST-MORTEM* EXAMINATION RESULTS**

RAT	COLON	GENERAL. P.M.	LUNGS	LIVER	PANCREAS	TESTES	OTHER
1	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
2	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
3	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
4	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	LESION ON RIGHT	NORMAL
5	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
6	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	LESION ON RIGHT	NORMAL
7	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
8	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
9	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
10	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	LESION ON RIGHT	MESENTERIC LESION
11	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
12	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
13	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
14	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	LESION ON LEFT	NORMAL
15	NORMAL	NORMAL	NODULAR	NORMAL	NORMAL	Tumour R Tumour L	NORMAL
16	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
17	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
18	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
19	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL
20	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	Tumour L	NORMAL

Total number of tissue abnormalities = 9

Percentage of animals with colonic diverticulosis = 0%

Table 5.2: This table illustrates *post-mortem* details for the group of familial high fibre fed rats. The condition of the heart, lungs, liver, pancreas and testes are shown, together with any other abnormalities. The incidence of colonic diverticulosis is shown together with the total number of tissue tumours or abnormalities.

P.M. REPORT SHEET

FAMILIAL LOW FIBRE GROUP

EXPERIMENTAL GROUP 1

POST-MORTEM **EXAMINATION RESULTS**

RAT	COLON	GENERAL. P.M.	LUNGS	LIVER	PANCREAS	TESTES	OTHER
1	Segmented	Small caecum	NORMAL	ABNORMAL	PALE, FATTY	NORMAL	BEZOAR IN STOMACH
2	Segmented	Small caecum	NORMAL	PALE	NORMAL	NORMAL	BEZOAR IN STOMACH
3	Segmented	Small caecum	NODULAR	PALE	NORMAL	Tumour L TumourR	BEZOAR IN STOMACH INFLAMED SEBACEOUS CYST
4	Segmented	Small caecum	NORMAL	PALE	NORMAL	Tumour L Tumour R	BEZOAR IN STOMACH
5	Segmented	Small caecum	NODULAR	PALE	NORMAL	NORMAL	BEZOAR IN STOMACH
6	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
7	Segmented	Small caecum	NORMAL	PALE	NODULAR	NORMAL	BEZOAR IN STOMACH
8	D.D. left colon	Small caecum	NORMAL	NORMAL	NODULAR	Tumour L	BEZOAR IN STOMACH SKIN LESION
9	—	—	—	—	—	—	—
10	D.D. left colon	Small caecum	Reticulated	PALE	NODULAR	NORMAL	BEZOAR IN STOMACH
11	D.D. mid colon	Small caecum	NORMAL	NODULAR TUMOUR	NODULAR	NORMAL	BEZOAR IN STOMACH
12	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
13	D.D. lower colon	Small caecum	NORMAL	NORMAL	NORMAL	Large tumour L	BEZOAR IN STOMACH
14	NORMAL	Small caecum	NORMAL	PALE	NODULAR	NORMAL	BEZOAR IN STOMACH
15	NORMAL	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH prominent peyers patches
16	D.D. mid colon	Small caecum	NORMAL	NORMAL	NORMAL	Tumour L Tumour R	BEZOAR IN STOMACH
17	—	—	—	—	—	—	—
18	D.D. mid colon	Small caecum	NORMAL	NORMAL	NODULAR	NORMAL	BEZOAIN STOMACH
19	—	—	—	—	—	—	—
20	D.D. mid colon	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH sebaceous skin cyst

— **Rat died prior to the termination of the experiment.**

Total number of tissue abnormalities = 48

Percentage of animals with colonic diverticulosis = 41.2%

Table 5.3: This table illustrates *post-mortem* details for the group of familial low fibre fed rats. The condition of the heart, lungs, liver, pancreas and testes are shown, together with any other abnormalities. The incidence of colonic diverticulosis is shown together with the total number of tissue tumours or abnormalities.

P.M. REPORT SHEET

WEANED LOW FIBRE GROUP

EXPERIMENTAL GROUP 2

POST-MORTEM EXAMINATION RESULTS

RAT	COLON	GENERAL. P.M.	LUNGS	LIVER	PANCREAS	TESTES	OTHER
1	D.D. mid colon	Small caecum	Reticulated	NORMAL	NODULAR	NORMAL	BEZOAR IN STOMACH
2	Segmented	Small caecum	NORMAL	PALE	NORMAL	NORMAL	BEZOAR IN STOMACH
3	D.D. mid colon	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
4	D.D. mid colon	Small caecum	NORMAL	PALE	NORMAL	NORMAL	BEZOAR IN STOMACH NODULE UPPER ABDOMEN
5	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
6	Segmented	Small caecum	NORMAL	PALE	NORMAL	NORMAL	BEZOAR IN STOMACH ABDOMINAL NODULE
7	——	——	——	——	——	——	——
8	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
9	Segmented	Small caecum	NORMAL	PALE	NORMAL	NORMAL	BEZOAR IN STOMACH
10	D.D. mid colon	Small caecum	NORMAL	PALE	Two, large nodules	NORMAL	BEZOAR IN STOMACH
11	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
12	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
13	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
14	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
15	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
16	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
17	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH
18	Segmented	Small caecum	NORMAL	NORMAL	NORMAL	NORMAL	BEZOAR IN STOAMCH ABDOMINAL LESION
19	Segmented	Small caecum	NORMAL	NORMAL	Small nodules	NORMAL	BEZOAR IN STOMACH MESENTERIC TUMOUR
20	Segmented	Small caecum	NODULAR	NORMAL	NORMAL	NORMAL	BEZOAR IN STOMACH

—— **Rat died prior to the termination of the experiment.**

Total number of tissue abnormalities = 34

Percentage of animals with colonic diverticulosis = 21.1%

Table 5.4 : This table illustrates *post-mortem* details for the group of weaned low fibre fed rats. The condition of the heart, lungs, liver, pancreas and testes are shown, together with any other abnormalities. The incidence of colonic diverticulosis is shown together with the total number of tissue tumours or abnormalities.

5.1.4 Total collagen content.

This involved the four designated regions of the colon together with the ileum and caecum. The total collagen content of the tissues was measured using the Chang *et al,* method (Chang K. *et al*, 1980). Results were obtained in triplicate and are expressed as median and range of the three results. The mean +/- standard deviation for the healthy and pathological tissue is shown. The results from these analyses are shown on Tables 5.5 - 5.7, and illustrated graphically on Graphs 5.2 - 5.4.

RAT NO.	ILEUM	CAECUM	ASCENDING	TRANSVERSE	DESCENDING	SIGMOID
1	13.3 (12.7-13.9)	13.0 (12.3-13.5)	13.3 (13.0-14.2)	13.4 (12.9-14.0)	13.7 (13.1-14.9)	13.2 (12.8-13.8)
2	13.5 (12.4-14.6)	12.6 (12.1-13.9)	13.3 (12.5-13.8)	13.9 (13.0-14.3)	13.3 (13.0-14.1)	14.0 (12.9-14.6)
3	13.6 (13.1-14.3)	12.9 (12.2-13.8)	13.6 (12.9-14.3)	13.4 (12.5-14.1)	13.7 (13.1-14.5)	13.3 (12.8-14.4)
4	13.5 (13.0-14.2)	13.1 (12.3-13.6)	13.6 (13.2-14.6)	13.2 (13.0-14.3)	13.3 (12.9-14.1)	13.3 (13.0-14.3)
5	13.6 (13.1-14.3)	13.3 (12.7-14.1)	13.5 (12.6-13.9)	13.6 (13.1-13.9)	13.9 (13.3-14.4)	13.8 (13.3-14.5)
6	13.7 (13.2-14.3)	13.2 (12.4-14.0)	13.1 (12.5-13.9)	13.6 (13.1-14.3)	13.6 (13.1-14.4)	13.8 (13.2-14.6)
7	13.0 (12.4-13.8)	13.3 (13.0-14.2)	13.3 (13.0-14.2)	13.5 (13.1-14.5)	13.3 (12.8-14.0)	13.3 (13.0-13.5)
8	13.2 (12.5-14.3)	13.1 (13.0-14.0)	13.5 (13.2-14.5)	13.4 (13.1-14.2)	13.5 (13.0-14.3)	13.4 (13.0-14.0)
9	13.1 (13.0-14.2)	13.3 (13.0-14.1)	13.7 (13.2-14.3)	13.9 (13.5-14.2)	13.0 (12.8-13.7)	13.1 (12.5-13.6)
10	13.5 (13.1-14.3)	13.6 (13.1-14.3)	13.6 (13.2-14.0)	13.4 (13.1-14.0)	13.1 (12.8-13.5)	13.8 (13.3-14.4)
11	13.1 (12.6-13.5)	13.7 (13.3-14.0)	13.3 (13.0-14.0)	13.5 (12.9-14.2)	13.6 (13.1-14.2)	13.5 (13.1-13.9)
12	13.6 (13.0-14.3)	13.6 (13.2-14.3)	13.5 (13.2-14.6)	13.7 (13.1-14.5)	13.8 (13.6-14.3)	13.3 (12.6-13.8)
13	13.7 (13.2-14.5)	13.1 (12.6-13.5)	13.5 (13.1-14.1)	13.6 (13.2-14.0)	13.5 (13.1-13.9)	13.6 (13.1-14.2)
14	13.5 (13.1-14.3)	13.2 (13.0-13.8)	13.5 (13.0-14.0)	13.8 (13.3-14.3)	13.6 (13.3-14.8)	13.3 (13.0-14.2)
15	13.1 (13.0-14.2)	13.5 (13.2-13.9)	13.5 (13.3-14.2)	13.0 (12.3-13.5)	13.1 (12.6-13.7)	13.1 (13.0-14.2)
16	13.2 (12.7-13.6)	13.0 (12.5-13.7)	12.9 (12.6-13.8)	13.1 (12.7-13.7)	13.8 (13.2-14.0)	13.8 (13.5-14.3)
17	13.2 (13.0-14.0)	13.2 (13.0-13.9)	13.7 (13.4-14.1)	13.1 (13.0-13.6)	13.3 (13.1-14.1)	13.3 (12.9-13.8)
18	13.8 (13.4-14.3)	13.3 (13.0-13.9)	13.1 (12.6-13.4)	13.8 (13.3-14.1)	13.5 (13.2-13.9)	13.7 (13.2-14.7)
19	13.1 (12.6-13.4)	13.2 (13.0-14.0)	13.5 (13.2-14.2)	13.9 (13.2-14.3)	12.9 (12.6-14.0)	13.4 (13.0-13.7)
20	13.8 (13.6-14.0)	13.5 (13.1-14.1)	13.1 (12.9-14.2)	13.2 (13.0-14.0)	13.2 (12.7-13.8)	13.5 (13.1-14.0)
HEALTHY mean +/- s.d.	13.41 +/- 0.26	13.24 +/- 0.26	13.41 +/- 0.22	13.50 +/- 0.28	13.44 +/- 0.29	13.48 +/- 0.26
average variability	4.1% (2.0-5.3)	4.3% (2.2-5.2)	4.2% (1.9-5.0)	4.3% (1.9-5.2)	4.6% (1.8-5.5)	3.8% (2.0-4.9)

Table 5.5: This table illustrates the results of the measurement of the total collagen content of the colon wall of familial high fibre fed rats. Results are expressed as a percentage of wet weight tissue, as median and range of the three results. The mean +/- standard deviation (s.d.) of the mean for healthy and pathological colons is shown. The variability of the methodology is also shown.

RAT NO.	ILEUM	CAECUM	ASCENDING	TRANSVERSE	DESCENDING	SIGMOID
1	13.2 (12.5-13.9)	12.9 (12.1-13.7)	13.2 (12.7-13.7)	13.3 (13.0-14.0)	13.6 (13.1-14.3)	13.1 (12.7-13.6)
2	13.4 (12.8-13.8)	12.5 (12.1-13.0)	13.2 (12.4-13.8)	13.8 (13.2-14.4)	13.2 (12.5-13.6)	13.9 (13.0-14.6)
3	13.5 (13.0-14.4)	12.8 (12.3-13.9)	13.5 (13.1-14.4)	13.3 (12.9-13.8)	13.6 (13.1-14.3)	13.2 (13.0-14.1)
4	13.4 (13.0-14.2)	13.0 (12.6-13.6)	13.5 (13.1-14.2)	13.1 (12.3-13.6)	13.2 (12.5-13.8)	13.2 (12.6-13.6)
5	13.5 (12.9-13.9)	13.2 (12.3-13.7)	13.4 (12.6-14.1)	13.5 (12.7-13.9)	13.8 (12.4-14.2)	13.7 (12.6-14.4)
6	13.6 (12.9-14.4)	13.1 (12.5-13.9)	13.0 (12.3-14.0)	13.5 (13.0-14.3)	13.5 (12.8-14.4)	13.7 (13.0-14.5)
7	12.9 (12.2-13.8)	13.4 (12.8-13.9)	13.2 (12.6-14.2)	13.4 (12.8-13.9)	13.2 (12.5-14.0)	13.2 (12.4-13.9)
8	13.1 (12.4-13.8)	13.0 (12.2-14.0)	13.4 (12.4-13.8)	13.3 (12.6-14.1)	13.4 (12.6-13.9)	13.3 (12.9-14.5)
9	—	—	—	—	—	—
10	13.4 (12.3-13.9)	13.5 (13.0-14.5)	13.5 (12.6-14.1)	13.3 (12.6-14.1)	13.0 (12.2-14.1)	13.7 (13.0-14.4)
11	13.0 (12.3-14.2)	13.6 (13.0-14.3)	13.2 (12.8-14.4)	13.4 (13.0-14.0)	13.5 (13.0-14.3)	13.4 (12.8-14.3)
12	13.5 (12.8-14.4)	13.5 (13.0-14.3)	13.4 (12.8-14.6)	13.6 (13.0-14.6)	13.7 (13.0-14.6)	13.2 (12.5-13.9)
13	13.6 (13.0-14.2)	13.0 (12.2-13.5)	13.4 (12.8-13.9)	13.5 (12.4-14.0)	13.4 (13.0-14.7)	13.5 (13.0-14.6)
14	13.4 (12.3-14.2)	13.1 (12.2-13.8)	13.4 (13.0-14.5)	13.7 (13.0-14.8)	13.5 (12.5-14.6)	13.2 (12.7-14.0)
15	13.0 (12.5-14.0)	13.4 (12.6-14.1)	13.4 (13.0-14.4)	12.9 (12.3-13.8)	13.0 (12.2-14.1)	13.0 (12.7-14.2)
16	13.1 (12.3-13.8)	12.9 (12.1-13.6)	12.8 (12.0-13.9)	13.0 (12.8-14.0)	13.7 (13.0-14.3)	13.7 (13.1-14.4)
17	—	—	—	—	—	—
18	13.7 (13.1-14.5)	13.2 (12.6-14.1)	13.0 (12.1-13.9)	13.7 (12.9-14.9)	13.4 (12.5-14.1)	13.6 (13.0-14.8)
19	—	—	—	—	—	—
20	13.7 (13.0-14.2)	13.4 (13.0-14.5)	13.0 (12.3-13.8)	13.1 (12.4-14.0)	13.1 (12.8-14.1)	13.4 (13.0-14.1)
HEALTHY mean +/- s.d.	13.34 +/- 0.23	13.09 +/- 0.31	13.32 +/- 0.16	13.41 +/- 0.27	13.43 +/- 0.26	13.34 +/- 0.31
DIVERTIC. mean +/- s.d.	13.37 +/- 0.30	13.23 +/- 0.28	13.19 +/- 0.26	13.33 +/- 0.24	13.36 +/- 0.24	13.51 +/- 0.16
average variability	4.0% (2.6-4.4)	4.2% (2.6-4.6)	4.4% (2.4-4.7)	4.3% (2.0-4.8)	4.2% (3.0-4.6)	4.1% (2.7-4.5)

= presence of diverticulosis

— = rat died prior to the termination of the experiment

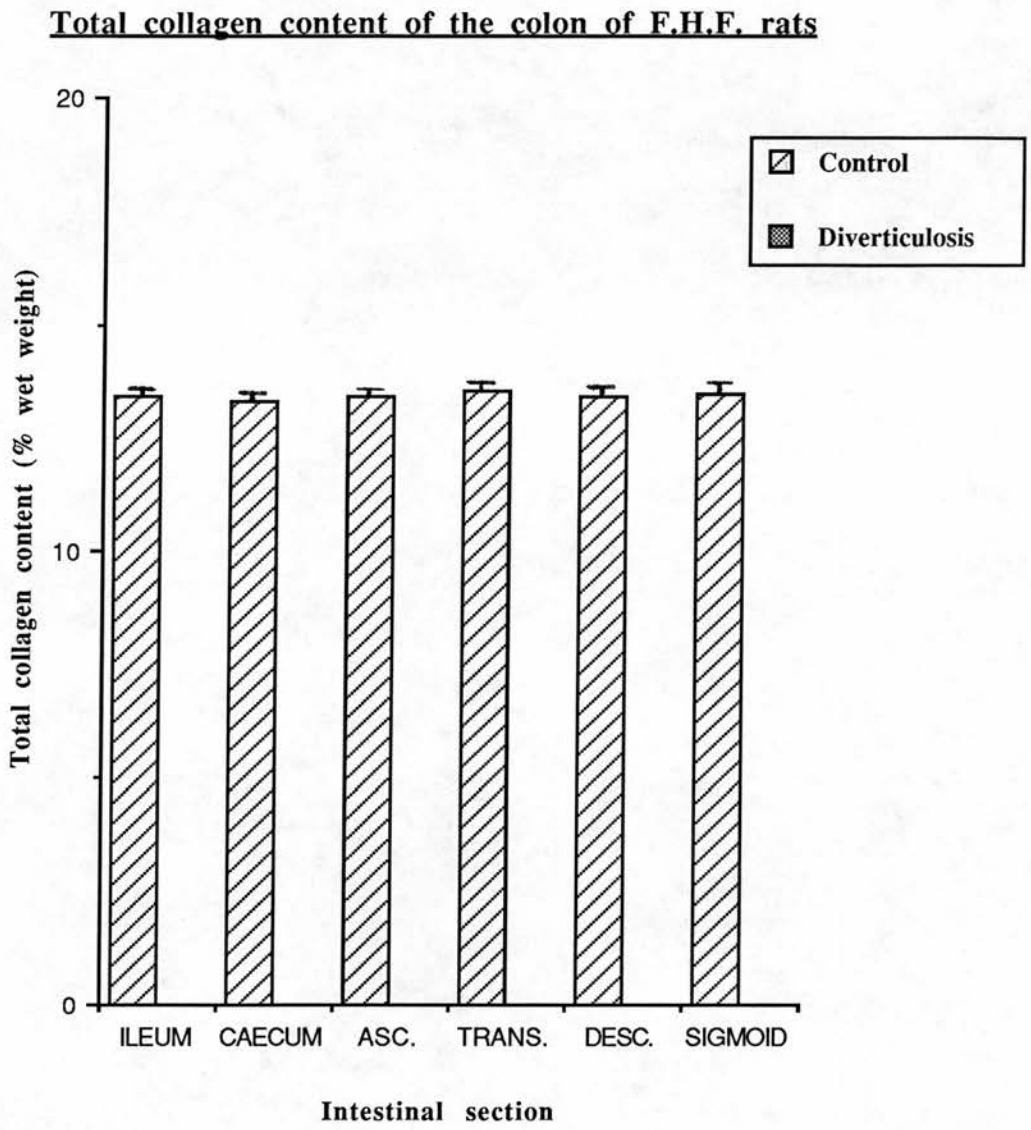
Table 5.6: This table illustrates the results of the measurement of the total collagen content of the colon wall of familial low fibre fed rats. Results are expressed as a percentage of wet weight tissue, as median and range of the three results. The mean +/- standard deviation (s.d.) of the mean for healthy and colonic diverticulosis affected colons is shown. The variability of the methodology is also shown.

RAT NO.	ILEUM	CAECUM	ASCENDING	TRANSVERSE	DESCENDING	SIGMOID
1	13.1 (12.8-13.2)	12.8 (12.6-13.1)	13.1 (12.9-13.4)	13.2 (13.0-13.5)	13.5 (13.1-13.9)	13.0 (12.8-13.3)
2	13.3 (12.9-13.5)	12.4 (12.1-12.8)	13.1 (12.7-13.4)	13.7 (13.5-14.0)	13.1 (12.8-13.5)	13.8 (13.5-14.0)
3	13.4 (13.1-13.9)	12.7 (12.2-13.0)	13.4 (13.0-13.8)	13.4 (13.1-13.7)	13.5 (13.2-13.8)	13.1 (12.9-13.3)
4	13.3 (13.0-13.7)	12.9 12.6-13.3)	13.4 (13.0-13.9)	13.0 (12.8-13.5)	13.1 (12.8-13.5)	13.1 (12.8-13.5)
5	13.4 (13.1-13.7)	13.1 (12.8-13.5)	13.3 (13.0-13.7)	13.4 (13.1-13.7)	13.7 (13.3-14.0)	13.6 (13.2-13.9)
6	13.5 (13.3-13.8)	13.0 (12.8-13.3)	12.9 (12.6-13.1)	13.4 (13.1-13.9)	13.4 (13.1-13.9)	13.6 (13.2-14.0)
7	—	—	—	—	—	—
8	13.0 (12.7-13.5)	12.9 (12.5-13.2)	13.3 (13.0-13.8)	13.2 (12.9-13.6)	13.3 (13.0-13.9)	13.2 (12.8-13.7)
9	13.2 (12.9-13.5)	13.5 (13.1-13.9)	13.6 (13.1-13.9)	13.8 (13.2-14.1)	13.9 (13.3-14.2)	12.9 (12.3-13.4)
10	13.3 (13.0-13.8)	13.4 (12.9-13.9)	13.4 (13.0-13.9)	13.2 (12.8-13.9)	12.9 (12.6-13.5)	13.6 (13.2-14.0)
11	12.9 (12.3-13.4)	13.5 (13.1-14.1)	13.1 (12.7-13.6)	13.3 (13.0-13.9)	13.4 (13.0-13.9)	13.3 (12.9-13.9)
12	13.4 (12.9-14.0)	13.4 (13.0-13.9)	13.3 (12.9-13.8)	13.5 (13.0-14.0)	13.6 (13.1-14.2)	13.1 (12.7-13.7)
13	13.5 (13.0-14.1)	12.9 (12.6-13.6)	13.3 (13.0-13.9)	13.4 (13.0-14.0)	13.3 (12.8-13.8)	13.4 (13.0-13.9)
14	13.3 (12.9-13.9)	13.0 (12.6-13.7)	13.3 (12.8-13.9)	13.6 (13.1-14.1)	13.4 (13.0-13.9)	13.1 (12.6-13.8)
15	12.9 (12.4-13.7)	13.3 (12.3-13.9)	13.3 (12.7-13.9)	12.8 (12.2-13.8)	12.9 (12.2-13.4)	12.9 (12.5-13.8)
16	13.0 (12.5-13.7)	12.8 (12.2-13.3)	12.7 (12.1-13.6)	12.9 (12.2-13.7)	13.6 (13.1-13.9)	13.6 (13.0-14.4)
17	13.3 (12.8-13.6)	13.5 (13.0-14.3)	13.4 (13.0-14.2)	13.1 (12.6-13.9)	13.2 (12.8-13.8)	13.1 (12.7-13.7)
18	13.6 (13.1-14.3)	13.1 (12.7-14.0)	12.9 (12.6-13.5)	13.6 (13.1-14.4)	13.3 (13.0-13.9)	13.3 (12.6-13.9)
19	13.4 (12.8-14.2)	13.6 (13.0-14.6)	13.8 (13.1-14.4)	13.4 (12.9-13.9)	13.3 (12.8-13.9)	13.1 (12.7-13.7)
20	13.6 (13.1-14.8)	13.3 (12.9-13.5)	12.9 (12.2-13.6)	13.0 (12.5-13.7)	13.0 (12.5-13.9)	13.3 (12.4-13.7
HEALTHY mean +/- s.d.	13.29 +/- 0.24	13.15 +/- 0.33	13.21 +/- 0.29	13.34 +/- 0.29	13.36 +/- 0.26	13.29 +/- 0.27
DIVERTIC. mean +/- s.d.	13.28 +/- 0.13	12.95 +/- 0.30	13.33 +/- 0.15	13.20 +/- 0.16	13.25 +/- 0.30	13.20 +/- 0.27
average variability	3.0% (2.7-3.3)	3.2% (2.8-3.6)	3.1% (2.7-3.5)	3.5% (2.7-3.7)	3.4% (3.1-3.7)	3.2% (2.4-3.7)

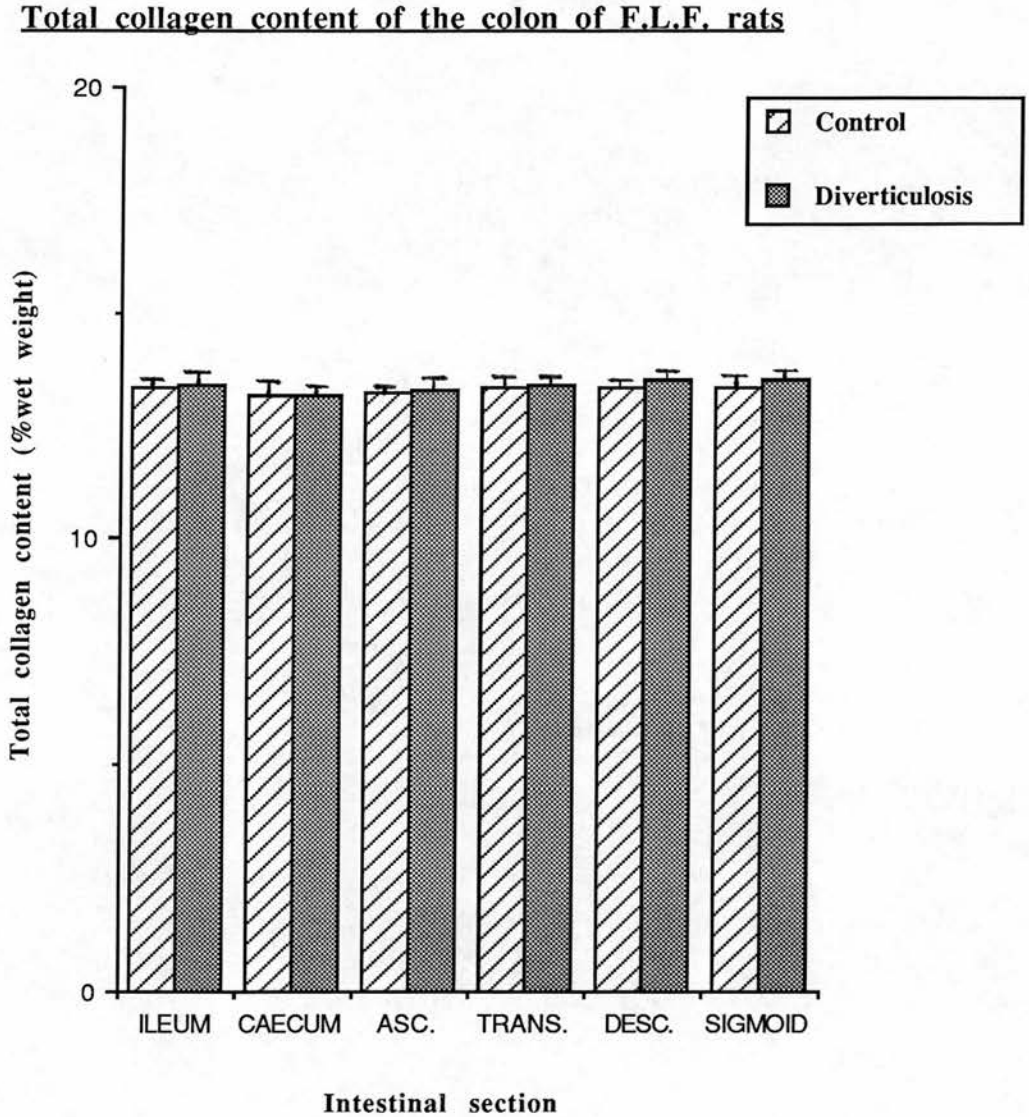
= presence of diverticulosis

— = rat died prior to the termination of the experiment

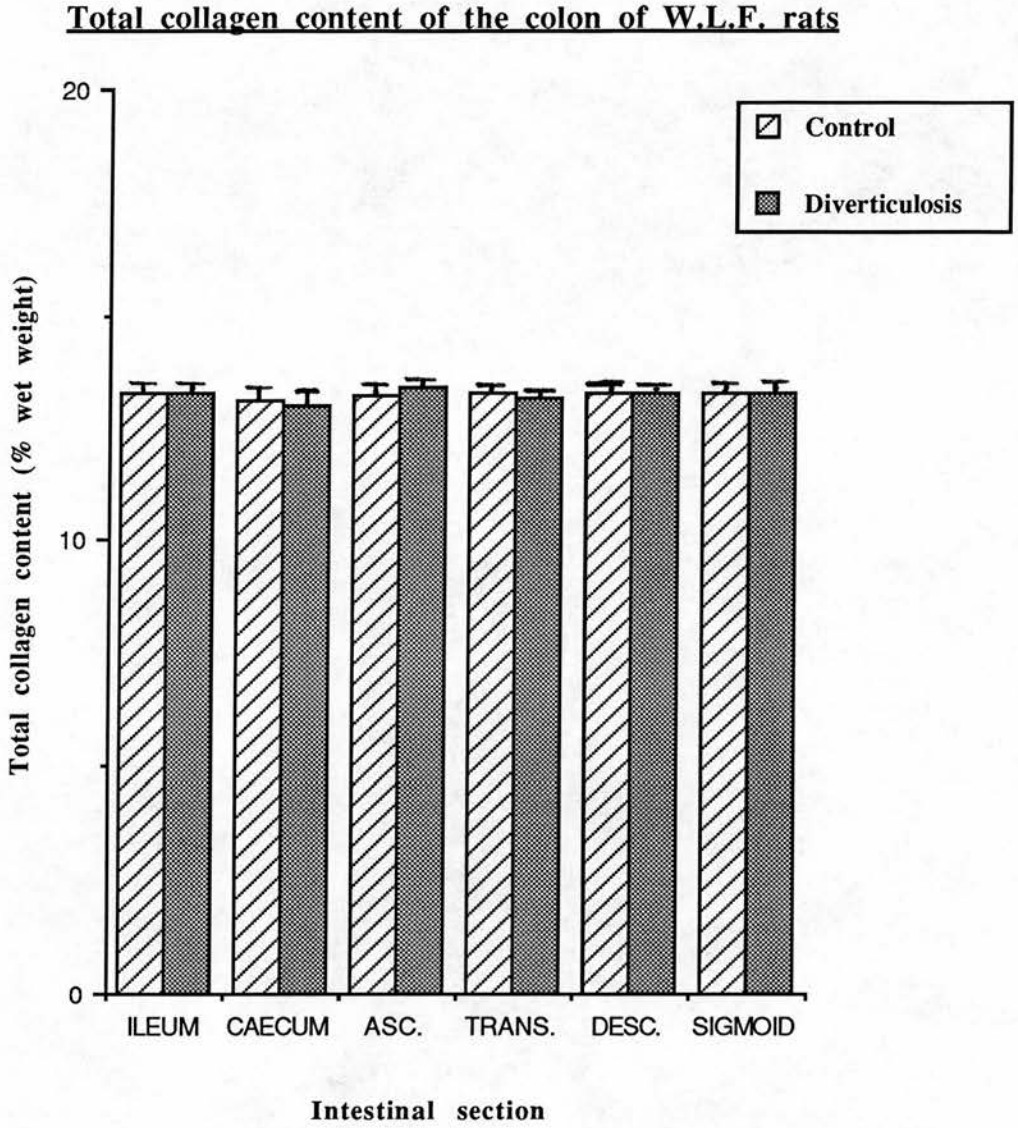
Table 5.7 : This table illustrates the results of the measurement of the total collagen content of the colon wall of weaned low fibre fed rats. Results are expressed as a percentage of wet weight tissue, as median and range of the three results. The mean +/- standard deviation (s.d.) of the mean of healthy and colonic diverticulosis affected colons is shown. The variability of the methodology is also shown.



Graph 5.2: This graph illustrates the results for the measurement of total collagen content of the colon wall from familial high fibre fed rats. Values are expressed as a percentage of the wet weight of the colon wall and are shown as mean +/- standard deviation (s.d.) of the mean. (▨) represents normal, healthy colons, and (■) those affected by colonic diverticulosis.



Graph 5.3: This graph illustrates the results for the measurement of total collagen content of the colon wall from familial low fibre fed rats. Values are expressed as a percentage of the wet weight of the colon wall and are shown as mean +/- standard deviation (s.d.) of the mean. (▨) represents normal, healthy colons, and (■) those affected by colonic diverticulosis.



Graph 5.4: This graph illustrates the results for the measurement of total collagen content of the colon wall from weaned low fibre fed rats. Values are expressed as a percentage of the wet weight of the colon wall and are shown as mean +/- standard deviation (s.d.) of the mean. (▨) represents normal, healthy colons, and (■) those affected by colonic diverticulosis.

5.1.5 Results of the measurement of the acid solubility of rat colon collagen.

The acid solubility of the collagen of all of the colonic sections was measured using the methodology outlined in Chapter three, section 3.6.1. Analysis was carried out in triplicate. The results are expressed as a ratio of acid insoluble to soluble collagen, as median and range of the three results. The mean +/- standard deviation of the mean are shown for the healthy and pathological colons. These results are illustrated in Tables 5.8 - 5.10, and graphically in Graphs 5.5 - 5.7.

RAT NO.	ILEUM	CAECUM	ASCENDING	TRANSVERSE	DESCENDING	SIGMOID
1	13.6 (13.3-14.4)	13.0 (12.7-13.7)	13.8 (13.2-14.2)	13.8 (13.3-14.5)	13.6 (13.2-14.0)	13.5 (13.0-14.0)
2	13.5 (13.1-14.1)	13.5 (12.9-14.3)	13.6 (13.1-14.5)	13.6 (13.1-14.4)	13.9 (13.1-14.3)	13.6 (13.0-14.3)
3	13.0 (12.4-13.6)	13.5 (13.1-14.2)	13.7 (13.3-14.6)	14.0 (13.4-14.7)	13.7 (13.1-14.3)	13.7 (13.2-14.0)
4	13.9 (13.2-14.1)	13.4 (13.1-13.9)	12.9 (12.4-13.5)	13.9 (13.6-14.3)	13.5 (13.2-14.5)	13.9 (13.4-14.6)
5	13.5 (13.2-14.6)	13.6 (13.2-14.8)	13.0 (13.0-13.7)	14.1 (13.6-14.5)	13.7 (13.4-14.3)	13.6 (13.2-14.4)
6	13.2 (13.0-14.0)	13.7 (13.4-14.1)	13.5 (13.1-14.0)	13.7 (13.2-14.3)	13.8 (13.5-14.5)	13.2 (13.0-13.9)
7	13.4 (13.1-14.1)	13.1 (13.0-13.8)	13.5 (12.5-13.4)	13.2 (13.1-14.0)	13.5 (13.2-13.9)	13.8 (13.3-14.0)
8	13.5 (13.1-13.9)	13.4 (13.0-14.2)	13.3 (12.7-13.9)	14.3 (13.4-15.0)	13.4 (13.0-14.3)	13.7 (13.2-14.3)
9	14.0 (13.2-14.8)	13.6 (13.1-14.5)	13.4 (13.0-13.9)	14.2 (13.3-14.6)	13.8 (13.4-14.6)	13.9 (13.4-14.7)
10	13.8 (13.1-14.3)	13.5 (13.4-14.4)	13.7 (13.2-14.5)	14.0 (13.0-14.3)	13.7 (13.4-14.1)	13.7 (13.5-14.1)
11	13.1 (13.0-14.0)	13.2 (13.1-13.9)	13.4 (13.2-13.9)	13.9 (13.0-14.4)	13.6 (13.2-13.9)	13.5 (13.1-14.1)
12	13.4 (13.0-13.9)	13.6 (13.3-14.1)	13.9 (13.4-14.5)	13.8 (13.2-14.3)	13.5 (13.2-14.0)	13.9 (13.6-14.3)
13	12.8 (12.7-13.9)	13.4 (13.2-13.8)	12.0 (12.0-13.4)	13.7 (13.6-14.1)	13.7 (13.3-14.1)	14.0 (13.6-14.5)
14	12.9 (12.7-13.8)	14.3 (14.1-14.9)	13.6 (13.3-14.1)	13.5 (13.2-13.9)	13.9 (13.8-14.7)	14.1 (14.0-14.9)
15	13.7 (13.4-14.3)	13.9 (13.7-14.5)	13.4 (13.2-13.8)	13.6 (13.2-14.1)	13.8 (13.2-14.3)	13.7 (13.5-14.5)
16	13.6 (13.4-14.4)	12.9 (12.6-13.8)	13.7 (13.2-14.0)	13.4 (13.0-14.1)	14.0 (13.4-14.8)	13.2 (13.0-13.8)
17	13.8 (13.1-14.5)	13.0 (12.7-13.4)	13.9 (13.4-14.5)	13.7 (13.6-14.6)	14.1 (13.7-14.6)	13.5 (13.1-14.2)
18	13.7 (13.2-14.2)	13.1 (13.0-13.7)	13.5 (13.2-13.8)	13.6 (13.2-14.2)	14.3 (14.1-14.9)	13.6 (13.0-14.3)
19	13.3 (13.0-14.2)	13.4 (13.1-14.0)	13.7 (13.2-13.9)	14.0 (13.5-14.6)	13.9 (13.4-14.3)	13.5 (13.1-14.1)
20	13.4 (13.1-13.9)	13.3 (12.6-13.8)	13.1 (13.0-14.3)	13.9 (13.5-14.3)	13.8 (13.4-14.3)	14.0 (13.5-14.5)
HEALTHY mean +/- s.d.	13.46 +/- 0.33	13.42 +/- 0.33	13.43 +/- 0.43	13.70 +/- 0.27	13.76 +/- 0.22	13.68 +/- 0.25
average variability	4.3% (1.7-5.2)	4.1% (1.5-4.6)	4.2% (1.6-5.0)	4.4% (1.8-6.3)	4.3% (1.9-5.7)	3.7% (2.0-4.8)

= presence of diverticulosis

—— = rat died prior to the termination of the experiment

Table 5.8: This table illustrates the results for the measurement of the acid solubility of colon collagen from familial high fibre fed rats (F.H.F.). Results are expressed as a ratio of insoluble : soluble collagen and are shown as median and range of the three results. The mean +/- standard deviation (s.d.) of the mean for healthy and pathological colons is shown. The variability of the methodology is also shown.

RAT NO.	ILEUM	CAECUM	ASCENDING	TRANSVERSE	DESCENDING	SIGMOID
1	15.7 (14.8-15.9)	17.5 (17.0-18.3)	21.3 (21.0-22.3)	24.2 (24.0-24.9)	26.9 (26.0-27.4)	30.2 (29.1-31.1)
2	15.2 (14.7-15.9)	17.2 (16.5-18.0)	22.1 (21.4-23.2)	24.1 (23.0-25.3)	27.0 (26.2-28.2)	31.3 (30.1-32.3)
3	14.9 (14.0-15.9)	17.8 (17.0-18.2)	22.0 (21.1-22.3)	24.0 (23.1-24.9)	27.5 (26.5-28.4)	30.3 (29.1-31.3)
4	15.0 (14.3-15.8)	16.8 (16.0-17.3)	21.6 (20.9-22.8)	24.0 (23.0-24.9)	27.5 (26.9-28.4)	31.6 (30.4-31.7)
5	14.8 (14.0-15.6)	16.9 (15.4-17.0)	21.7 (20.1-22.6)	23.8 (22.3-24.8)	28.1 (27.4-28.9)	31.9 (30.9-32.6)
6	15.3 (15.0-15.9)	17.9 (17.0-18.8)	21.3 (20.1-22.5)	23.9 (22.4-24.0)	26.9 (25.8-27.3)	30.7 (29.1-30.9)
7	15.9 (15.0-16.3)	17.1 (16.3-17.5)	21.6 (20.3-22.3)	23.1 (22.1-24.3)	27.3 (26.0-27.7)	30.8 (29.3-30.9)
8	18.6 (17.8-18.8)	19.4 (18.7-19.7)	23.4 (22.3-24.4)	27.3 (26.8-27.6)	30.2 (29.0-31.6)	36.6 (34.2-37.0)
9	—	—	—	—	—	—
10	18.9 (17.9-19.1)	19.7 (18.6-19.9)	25.1 (24.0-25.7)	27.5 (25.9-27.8)	30.1 (28.6-30.5)	36.6 (34.4-37.1)
11	15.8 (15.2-16.5)	17.3 (16.5-17.9)	21.8 (20.5-22.0)	22.9 (21.3-23.4)	27.3 (26.1-28.4)	32.3 (30.5-33.0)
12	18.2 (17.5-18.9)	19.9 (18.4-20.3)	25.7 (24.3-26.3)	27.9 (26.7-30.0)	30.8 (28.8-31.2)	36.1 (34.4-37.2)
13	18.4 (17.7-18.9)	19.8 (18.8-20.1)	25.5 (24.4-26.7)	27.6 (26.5-28.4)	30.4 (28.3-31.9)	36.6 (34.2-38.4)
14	15.5 (15.0-16.3)	17.3 (16.5-17.4)	22.6 (21.3-24.5)	23.5 (22.4-24.6)	26.7 (25.7-28.0)	31.2 (28.5-32.6)
15	16.0 (15.5-16.7)	17.8 (17.0-18.9)	21.9 (20.8-22.3)	22.5 (21.6-24.0)	26.9 (26.4-28.2)	32.1 (31.3-33.5)
16	18.2 (17.5-19.1)	19.9 (18.9-20.3)	25.6 (24.3-26.5)	27.9 (25.6-28.5)	30.0 (28.7-31.3)	36.7 (35.6-38.0)
17	—	—	—	—	—	—
18	18.6 (18.0-19.8)	19.3 (18.9-20.9)	25.3 (24.3-26.9)	27.2 (25.2-28.8)	30.2 (28.5-31.3)	36.9 (35.4-38.2)
19	—	—	—	—	—	—
20	18.8 (17.8-19.8)	19.9 (18.7-20.9)	25.1 (23.7-26.5)	27.2 (26.5-28.9)	30.5 (28.6-31.3)	36.5 (35.5-37.9)
HEALTHY mean +/- s.d.	15.41 +/- 0.43	17.36 +/- 0.38	21.79 +/- 0.39	23.60 +/- 0.56	27.21 +/- 0.42	31.24 +/- 0.74
DIVERTIC. mean +/- s.d.	18.53 +/- 0.28	19.7 +/- 0.25	25.10 +/- 0.79	30.03 +/- 0.27	30.03 +/- 0.27	36.6 +/- 0.24
average variability	4.1% (1.8-6.5)	4.7% (2.1-7.0)	4.1% (2.2-5.8)	4.4% (2.1-6.5)	4.8% (1.9-6.9)	5.2% (2.3-7.5)

= presence of diverticulosis

— = rat died prior to the termination of the experiment

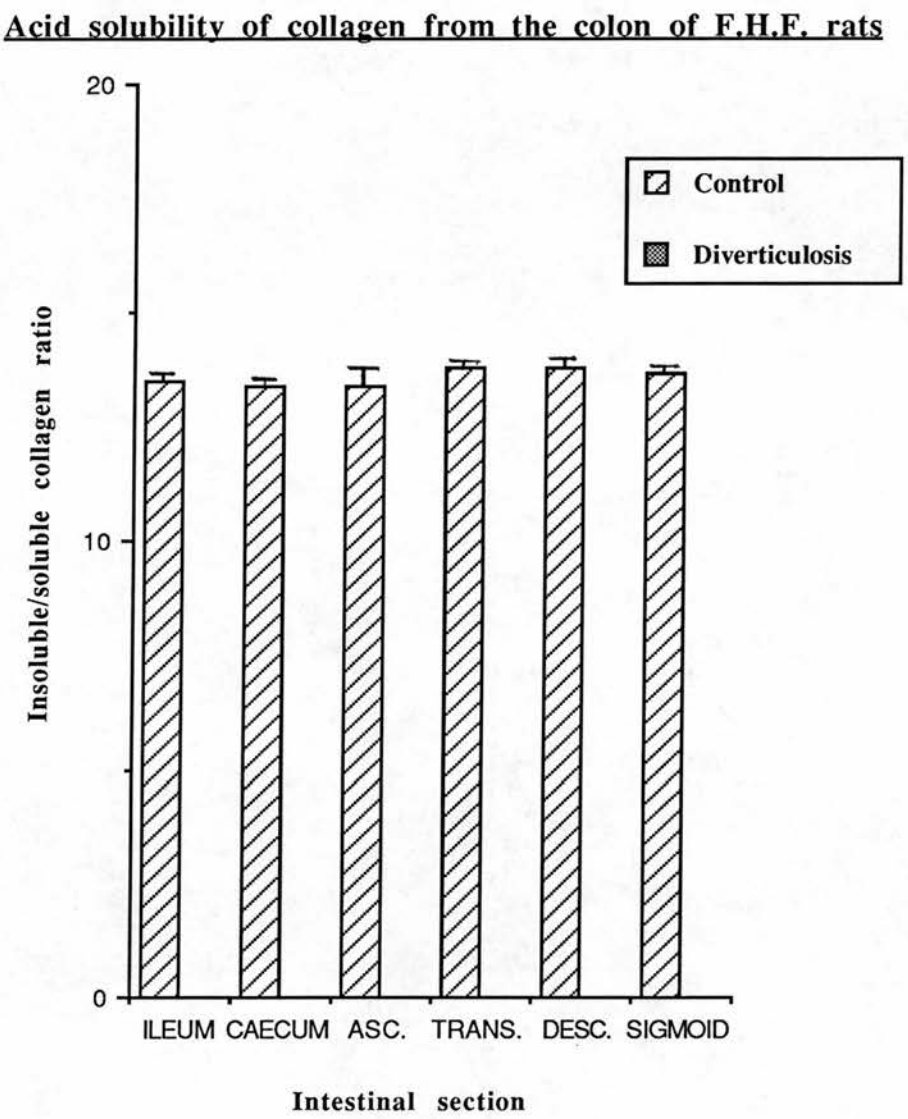
Table 5.9: This table illustrates the results for the measurement of the acid solubility of colon collagen from familial low fibre fed rats (F.L.F.). Results are expressed as a ratio of insoluble : soluble collagen and are shown as median and range of the three results. The mean +/- standard deviation (s.d.) of the mean for healthy and pathological colons is shown. The variability of the methodology is also shown.

RAT NO.	ILEUM	CAECUM	ASCENDING	TRANSVERSE	DESCENDING	SIGMOID
1	17.8 (17.0-18.7)	19.7 (18.7-20.4)	20.5 (19.2-21.5)	23.1 (22.0-24.3)	26.7 (25.6-27.9)	30.9 (29.7-31.4)
2	14.2 (13.7-14.6)	15.0 (14.5-15.9)	17.2 (16.2-17.9)	18.7 (17.9-19.3)	19.2 (18.6-19.9)	23.4 (22.4-24.1)
3	17.1 (16.4-17.8)	19.5 (18.5-20.8)	21.3 (20.2-21.9)	23.8 (22.8-24.9)	25.0 (23.8-26.0)	29.2 (28.0-30.4)
4	17.9 (17.0-18.6)	19.8 (18.8-20.7)	20.5 (19.5-21.1)	22.9 (21.9-23.4)	25.4 (24.3-26.5)	29.9 (28.7-30.7)
5	14.6 (14.2-15.2)	14.2 (14.0-14.9)	17.0 (16.2-17.8)	18.0 (17.2-18.9)	18.9 (18.1-19.9)	23.5 (22.5-24.4)
6	13.9 (13.2-14.2)	14.8 (14.0-15.3)	17.3 (16.7-17.9)	18.6 (17.9-19.0)	19.7 (18.7-20.6)	22.5 (21.6-23.3)
7	—	—	—	—	—	—
8	14.6 (13.9-14.9)	15.7 (15.0-16.3)	17.1 (16.5-18.2)	18.7 (18.0-19.7)	19.0 (18.1-19.7)	21.6 (20.7-22.2)
9	14.5 (14.0-15.0)	15.3 (15.0-16.0)	16.9 (16.2-17.4)	17.9 (17.1-18.9)	19.1 (18.0-19.4)	21.8 (20.9-22.3)
10	17.9 (17.0-18.3)	19.9 (18.9-20.3)	21.0 (20.3-21.9)	22.5 (21.5-22.9)	26.4 (25.3-27.8)	29.8 (28.8-30.9)
11	13.6 (13.3-14.0)	15.2 (14.8-15.7)	16.8 (15.8-17.1)	18.6 (17.6-18.9)	19.7 (18.6-19.9)	22.1 (21.1-22.9)
12	14.1 (13.8-14.5)	15.0 (14.5-15.5)	17.5 (16.7-18.2)	17.4 (16.8-17.9)	18.9 (17.9-19.7)	22.4 (21.4-22.9)
13	14.0 (13.7-14.9)	14.7 (14.4-15.0)	17.2 (16.5-17.9)	18.6 (17.6-19.5)	18.8 (17.8-19.5)	22.6 (21.9-23.3)
14	14.3 (14.0-14.7)	14.0 (13.6-14.5)	17.1 (16.5-17.9)	17.5 (16.8-18.0)	19.4 (18.8-20.0)	23.7 (22.7-24.7)
15	14.6 (14.3-14.9)	15.4 (15.0-15.9)	17.6 (16.8-18.2)	18.4 (17.4-19.4)	19.4 (19.0-20.3)	24.1 (23.0-25.3)
16	13.9 (13.5-14.3)	14.5 (14.2-14.9)	18.0 (17.4-18.9)	17.8 (16.8-18.6)	19.0 (18.1-19.9)	23.7 (22.6-24.5)
17	13.5 (13.1-14.0)	14.7 (14.2-15.2)	16.9 (15.9-16.8)	18.2 (17.2-18.9)	18.9 (17.9-19.8)	21.4 (20.6-22.3)
18	14.8 (14.4-15.2)	14.9 (14.5-15.3)	17.5 (16.8-18.2)	17.9 (16.9-18.5)	19.7 (18.7-20.2)	22.9 (21.9-23.5)
19	14.5 (14.2-14.9)	14.9 (14.3-15.6)	17.4 (17.0-18.4)	18.0 (17.2-18.7)	19.2 (18.3-19.9)	23.1 (22.2-24.8)
20	13.9 (13.1-14.1)	14.0 (13.5-14.3)	17.3 (16.9-17.9)	18.7 (17.9-19.4)	19.0 (18.2-19.9)	23.4 (22.4-25.0)
HEALTHY mean +/- s.d.	14.20 +/- 0.40	14.82 +/- 0.49	17.25 +/- 0.32	18.20 +/- 0.45	19.19 +/- 0.32	22.81 +/- 0.84
DIVERTIC. mean +/- s.d.	17.7 +/- 0.39	19.7 +/- 0.17	20.83 +/- 0.39	23.08 +/- 0.54	25.88 +/- 0.81	29.95 +/- 0.71
average variability	4.2% (2.0-6.7)	4.0% (2.0-6.1)	4.3% (2.3-6.1)	4.6% (1.9-6.9)	4.4% (2.4-6.5)	4.2% (2.4-6.6)

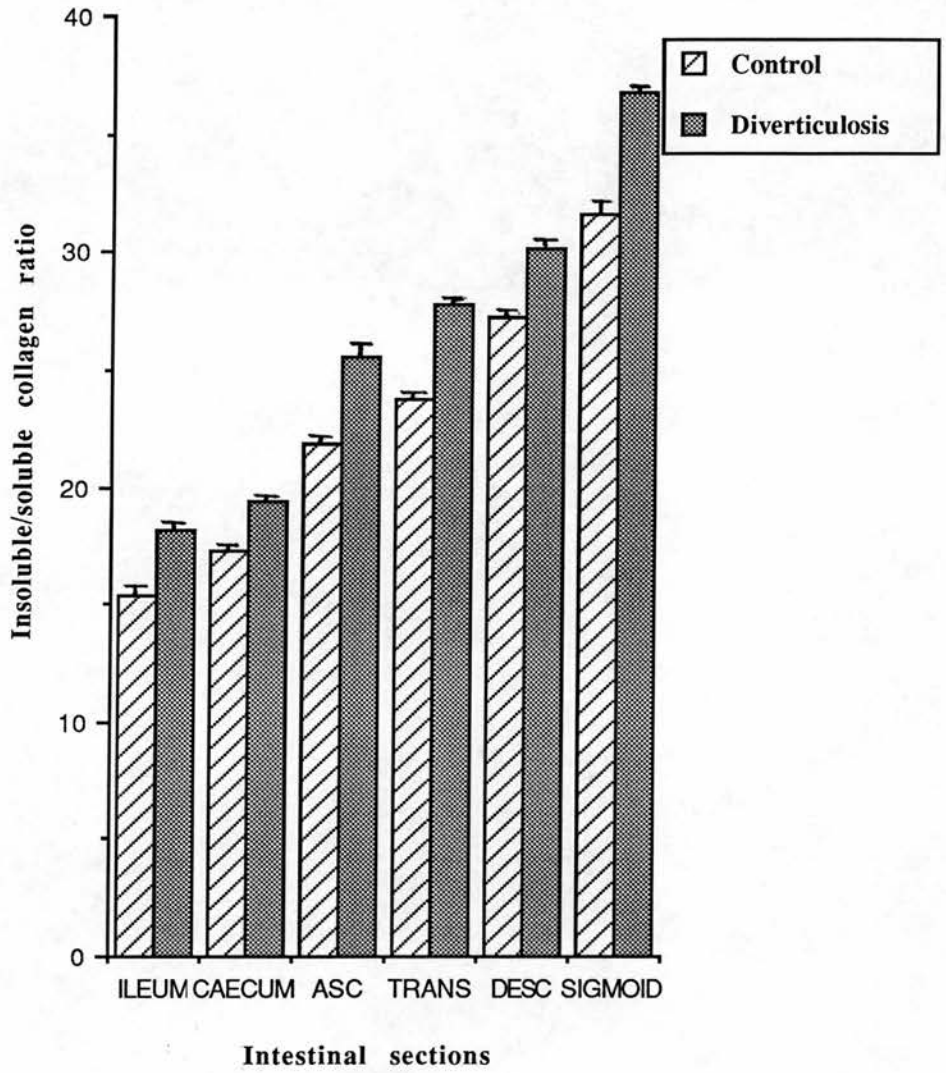
= presence of diverticulosis

— = rat died prior to the termination of the experiment

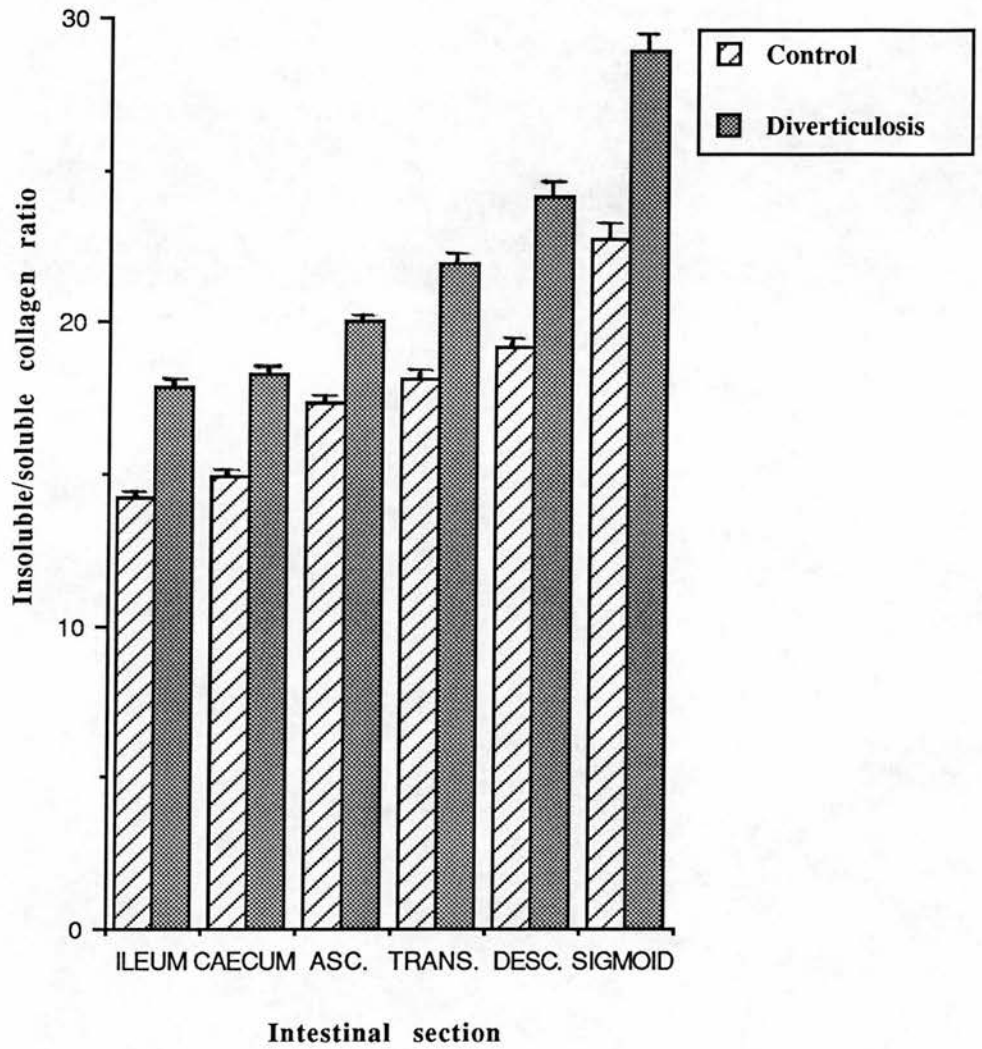
Table 5.10: This table illustrates the results for the measurement of the acid solubility of colon collagen from weaned low fibre fed rats (W.L.F.). Results are expressed as a ratio of insoluble : soluble collagen and are shown as median and range of the three results. The mean +/- standard deviation (s.d.) of the mean for healthy and pathological colons is shown. The variability of the methodology is also shown.



Graph 5.5: This graph illustrates the results for the measurement of the acid solubilty of colon collagen from the familial high fibre fed rats (F.H.F.). Values are expressed as a ratio of insoluble : soluble collagen as mean +/- standard deviation (s.d.) of the mean. (▨) represents normal, healthy colons and (■) those affected by colonic diverticulosis.



Graph 5.6: This graph illustrates the results for the measurement of the acid solubility of colon collagen from the familial low fibre fed rats (F.L.F.). Values are expressed as a ratio of insoluble : soluble collagen as mean +/- standard deviation (s.d.) of the mean. (▨) represents normal, healthy colons and (■) those affected by colonic diverticulosis.



Graph 5.7: This graph illustrates the results for the measurement of the acid solubility of colon collagen from the weaned low fibre fed rats (W.L.F.). Values are expressed as a ratio of insoluble : soluble collagen as mean +/- standard deviation (s.d.) of the mean. ( ) represents normal, healthy colons and ( ) those affected by colonic diverticulosis.

Sample	Mean	standard deviation	standard error	p value
F.L.F. ascending total collagen v	13.32	0.23	0.05	non significant
F.L.F. sigmoid total collagen.	13.34	0.31	0.09	
F.H.F. ascending total collagen v	13.41	0.22	0.05	non significant
F.H.F. sigmoid total collagen.	13.48	0.26	0.06	
W.L.F. ascending total collagen v	13.21	0.29	0.07	non significant
W.L.F. sigmoid total collagen.	13.29	0.27	0.07	
F.L.F. ascending solubility ratio v	21.79	0.39	0.12	p<0.001
F.L.F. sigmoid solubility ratio.	31.24	0.74	0.23	
F.H.F. ascending solubility ratio v	13.43	0.43	0.09	non significant
F.H.F. sigmoid solubility ratio.	13.68	0.25	0.05	
W.L.F. ascending solubility ratio v	17.25	0.32	0.08	p<0.001
W.L.F. sigmoid solubility ratio.	22.81	0.84	0.22	
F.L.F. ascending solubility ratio (N) v	21.79	0.39	0.12	p<0.001
F.L.F. ascending solubility ratio (D).	25.10	0.79	0.30	
W.L.F. ascending solubility ratio (N) v	17.25	0.32	0.08	p<0.001
W.L.F. ascending solubility ratio (D).	20.83	0.39	0.20	
F.L.F. sigmoid solubility ratio (N) v	31.24	0.74	0.23	p<0.001
F.L.F. sigmoid solubility ratio (D).	36.60	0.24	0.09	
W.L.F. sigmoid solubility ratio (N) v	22.81	0.84	0.19	p<0.05
W.L.F. sigmoid solubility ratio (D).	29.95	0.71	0.36	

Table 5.11: This table illustrates the statistical analysis of the results from the dietary experiment. Comparisons are made between normal (N) colons and pathological (D) colons. Results are expressed as mean, standard deviation, standard error and the resultant p value after analysis.

5.2 Discussion of the results.

The results produced in this chapter have an important bearing on the aetiology of colonic diverticulosis. The results for the relationship between diet and the incidence of colonic diverticulosis substantiate the dietary fibre theory.

The most striking visual difference between the three groups of rats is illustrated by the growing pattern and the terminal body weights. The two low fibre diet fed groups are significantly heavier than the high fibre diet fed group. They reached stable body weights more quickly than the low fibre diet fed rats.

A result of interest is the effect of maternal diet on the structure of colonic collagen. This is illustrated by the collagen solubility measurements. Collagen solubility is decreased in the F.L.F. compared with the F.H.F. and the W.L.F.. This could indicate that the collagen has been structurally altered in the developing young due to the maternal diet. The influence of maternal feeding and body weight in the infant, in relation to adult health is a very important area of study (Barker D.J.P., 1989).

Another significant result is the increase in the insoluble collagen in the colon wall from the right side to the left side i.e from the ascending and transverse regions to the descending and sigmoid regions. There are statistically significant differences between the different sites. This is of interest as the left colon is the predominant location for colonic diverticula in developed, elderly, western populations.

The difference between the number of tumours occurring in the three groups is very significant. The weaned low fibre group have a lower incidence of tumours than the familial low fibre group, although both groups were fed

identical diets. This indicates that the presence of dietary fibre in the maternal diet is protective against the development of tissue abnormalities, and potentially colonic diverticulosis.

The incidence of colonic diverticulosis is also significantly different between the three groups under study. The familial high fibre fed group had 0% incidence of colonic diverticula. The two groups fed a low fibre diet (F.L.F. and W.L.F.) had incidences of > 20%. It is of interest that there is a difference between the two low fibre groups. The familial low fibre group has a two fold higher incidence of colonic diverticulosis than the weaned group. This indicates that the presence of fibre in the maternal diet has a protective influence against colonic diverticulosis and perhaps related disorders. These results are paramount in examination of the developing young.

Chapter 6

Discussion of the methodology

and results.

6.1 Discussion of the methodology for the analysis of human tissue.

A structural study of any biological tissue calls for careful planning prior to tissue collection. This is to avoid autolysis of the tissue due to improper collection or storage. The tissue used in this study was all collected *post-mortem* and stored at -70^{0}C until analysis. All tissues were collected prior to 17 hours after death, to minimise autolysis of the tissue, and any *post-mortem* changes.

6.1.1 Subjects chosen.

All of the subjects involved in this study were chosen specifically by the consultant pathologist, in order that the cause of death was not related in any way to bowel disease. The subjects died of a variety of causes, as outlined in Chapter 3, Table 3.1. The various causes of death were considered by the pathologist not to affect the collagen of the body. The subjects were thought to be a representative cross-section of the population. The subjects judged as having colonic diverticulosis were diagnosed at *post-mortem* by the same consultant pathologist. Those subjects which were diagnosed as having a normal healthy bowel, had no evidence of colonic diverticulosis or other colonic disorders.

The subjects were selected over an age range of 20 years to 80 years (median 57 years, mean 55 years). This was judged as be an effective range in order to study the effects of age on the structure of the collagen of the colon. The subjects comprised 12 males and 13 females with an equal number of males and females in the aged and young categories. A previous T.E.M. study of the structure of collagen in the colon, observed no statistically significant differences between male and female subjects (Thomson H.J. *et al*, 1987b).

6.1.2 *Post-mortem* tissue.

Colonic tissue is not readily obtained from the living human. The only specimens available are those resected at operation for underlying bowel disease. These are usually of the older age group. *Post-mortem* tissue is the only efficient and ethical means of obtaining colonic tissue from unaffected bowel from a wide range of ages. *Post-mortem* tissue is less appropriate than fresh operative samples. The tissue has been deprived of its essential blood supply and there may be changes due to autolysis of the tissue. These effects can be kept to a minimum if the correct conditions are chosen. One study has indicated that there is no difference in measured parameters between operative and *post-mortem* samples, provided that the tissue was removed prior to 24 hours after death (Thomson H.J. *et al*, 1987b).

6.1.3 Time after death.

The time after death at which the tissue is removed is an important variable in tissue analysis. This study has therefore aimed to maintain this period to as short a time as possible (always before 17 hours after death). However, it is only an assumption that a short time after death is reasonable.

An investigation into the correlation of one of the measured parameters (in this case the collagen solubility) was made against time after death of the subject (results not shown). Results indicated that there was no correlation between time after death and autolysis of the tissue within the range of storage times used. The use of *post-mortem* tissue was therefore regarded as a viable study condition for the analysis of the biochemical and structural parameters of colonic collagen.

6.1.4 Storage of the tissue.

All cadavers were stored at 4^0C for the short interval prior to *post-mortem* examination. This was to reduce the effect of any *post-mortem* changes. After removal from the cadavers, the tissues used in this study were all flash frozen with liquid nitrogen prior to freezer storage. Flash freezing was used, as the effects of damage to the tissue by delayed freezing techniques could not be ruled out. The tissues were thereafter maintained in a -70^0C freezer until they could be analysed. They were maintained for no longer than three weeks. This length of time was considered to be reasonable for storage without damage to the tissue. It was considered that the storage of the samples did not affect the structural parameters under measurement. No previous differences have been observed with respect to changes in the signal to noise ratio of diffraction patterns of rat tail tendon after storage at -70^0C for up to six months. This observation can presumably be applied to colonic submucosa.

6.1.5 *Post-mortem* measurements.

The experiments contained in this thesis were designed with the purpose of examining the effects of both age and diet. *Post-mortem* measurements of colonic tissue samples were conducted in triplicate and expressed as median and range of the three results. Analysis in triplicate facilitated examination of the reproducibilty of the experimental procedure and also statistical analysis. The X-ray diffraction experiments were carried out on specimens from all four sections of representatives of the 25 colons under examination. This could only be carried out in duplicate due to the limited time which was available at the S.R.S., Daresbury, England, U.K.. This was considered to be adequate for this particular study.

In the case of single crystal diffraction and fibre diffraction, the data obtained from one sample alone is regarded as being sufficient (often optimal) to provide a structure. The error of data collection can be indicated in the lack of fit between observed and calculated data. From this, it is feasible to infer that the error of recording a reflection intensity is between 5 and 10 percent.

For the scope of this thesis, the measurement of collagen solubility in weak acid, was considered reasonable as an indicator for the analysis of the aging effect on the human colon. Age related changes in the reducible components of collagen have been detected (Robins S.P., Shimokomaki M. and Bailey A.J., 1973). The measurement of collagen solubility gives an indirect, inverse measure of the amount of crosslinks which are insoluble in weak acids. The insoluble residue can be regarded as due to ketoimine crosslinks, mature crosslinks of both the allysine and hydroxyallysine pathways, and advanced glycation products. The acid solubility of a crosslink in collagen depends on the presence of the crosslink in a Schiff base form.

Analysis of human colonic collagen by X-ray fibre diffraction is an ideal experimental system. The submucosa can be isolated from the surrounding muscle and fat and examined intact in a fully hydrated environment. Synchrotron radiation was considered the most useful source of X-rays, since it is the most brilliant and high flux X-ray source. Early studies of colon collagen in this thesis using a conventional rotating anode source were considered too damaging to the tissue. This was due to the exposure time being in the region of 100 hours. This meant that the sample dried out and incurred beam damage.

6.2 Discussion of the methodology for analysis of animal tissue.

The use of the laboratory rat as an experimental model for the study of diet as an aetiological factor in the development of human colonic diverticulosis has been criticised. This is largely on the basis of the distribution of diverticula in the rat compared with that of the human. In the rat, the region affected is the colon adjacent to the caecum. In the human, diverticulae are mainly seen in the sigmoid colon (Brodribb A.J.M., 1980), (Brodribb A.J.M., 1981). This is however, not a valid criticism of the use of animal tissue for the study of human colonic diverticulosis, as diverticulae in Japanese culture occur mainly in the ascending colon (Eastwood M.A., Eastwood J. and Ward M., 1976). Diverticulae have also been found in the right colon in other groups (Hughes L.E., 1969). The reason for these differences is not understood.

Fisher produced evidence that the rat was an appropriate model for the study of human colonic diverticulosis. The occurrence of lines of diverticulae in the rat were similar to those seen in human subjects (Fisher N. *et al*, 1985). Various groups have examined the use of laboratory animals as models for the study of human colonic diverticulosis. Some have examined the rabbit as a model for colonic diverticulosis, however these studies were uncontrolled and the results unspecific (Hodgson I., 1972), (Hodgson W.J.B., 1972). Previous experiments using the laboratory rat, (Lubbock D.M., Thomson W. and Garry R.C., 1937), (Carlson A.J. and Hoelzel F., 1949), (Morgan M.N. and Ellis H., 1969) were again uncontrolled and subject to criticism.

The dietary experiment carried out in this thesis was on a relatively small scale, although the experimental conditions were very well controlled. All of the rats selected for this study were bred solely for this purpose from the same stock animals, and breeding was carefully monitored. The diets which were used were all refined animal diets prepared to defined specifications. Diets differed

in the non-starch polysaccharide content. This difference was large enough to label the control group (F.H.F.) as high fibre, and the two experimental groups (F.L.F. and W.L.F.) as low fibre. The low fibre diet was used from one batch, to avoid differences between batches and was prepared meticulously each day. All tissue samples were removed at *post-mortem* immediately after death. This was thought to be a viable method for the study of colon collagen from laboratory rats as a model for the study of colonic diverticulosis in humans.

6.3 Discussion of results.

Several theories exist relating to the aetiology of colonic diverticulosis. This section of the discussion aims to examine the two major theories, how they conflict and how they are related. One of the first theories to be proposed, was that the increase in the incidence of the disease followed a fall in the consumption of dietary fibre during the 20th century (Painter N.S. and Burkitt D.P., 1975). This was the accepted theory for a number of years, supported by the observation that the symptoms of the condition are curbed by simply prescribing increased dietary fibre intake. Painter also examined the high intraluminal pressures associated with colonic diverticulosis (Painter N.S. and Truelove S.C., 1964). They related these to the increased pressure required to propel the small, dry stools which are inevitably produced on a diet which is low in dietary fibre.

The second theory described here is associated with changes in the structure and strength of the colon wall with aging. The mechanical properties of certain tissues have been shown to change with aging (Daly C.D. and Odland G.F., 1979), (Watters D.A.K., 1983). A more recent review has discussed the relationship between the strength of the colon wall and colonic diverticulosis. The proposal that the mechanical properties of the colon wall are a key factor in

the development of diverticulosis is discussed (Watters D.A.K. and Smith A.N., 1990). This review debates the significance of the observation that the colon is narrowest in its distal sigmoid region, and this region becomes narrower with advancing age (Watters D.A.K. *et al*, 1985). Narrowing of the colon would increase the intraluminal pressures in the colon and adversely affect the colon wall, which may be under increased pressure from a low residue diet.

6.3.1 The effect of age on the collagen of the human colon wall.

The experiments presented in this thesis attempt to examine the importance of a structural theory in relation to the aetiology of colonic diverticulosis. This thesis examines submucosal collagen, an important colonic wall component, providing the mechanical integrity of the colon wall. The development of colonic diverticulosis has been shown to be a consequence of advancing age. This is demonstrated by alteration of the collagen of the colon in both healthy and pathological tissues due to aging. The change in colonic collagen, took the form of an increase in the numbers of amino acid residues involved in acid stable covalent crosslinkage with adjacent residues. This was illustrated by a decrease in the solubility of colon collagen in weak acid.

Collagen crosslinks serve to stabilise the tissues in which they are found. However, if the degree of collagen crosslinkage increases continually, the resultant tissue then becomes more rigid (Schnider S.L. and Kohn R.R., 1982). The ratio of insoluble to soluble collagen, and hence the inferred level of mature and age related crosslinked collagen, is significantly different between age matched, healthy colons and colons with colonic diverticulosis. This indicates that aging is not the sole aetiological factor in the development of colonic diverticulosis. This experiment illustrates that there are no significant differences in the total collagen content or collagen solubility in the colon wall

between male and female subjects. This indicates that changes in the colon collagen, associated with the aging process are occurring in both the male and female population to the same extent.

Recent work on colonic diverticulosis has concentrated on the treatment of the symptoms using a variety of methods, from dietary fibre, to medication and ultimately to surgery. The condition would benefit from experiments which examine prevention of the condition or at least the symptoms which arise as a consequence. The population should attempt to remain pain and symptom free by consuming a diet rich in fibre and reducing the pressures within the colon.

This thesis has produced structural evidence that colonic diverticula develop as a consequence of aging in the elderly western population. In all four sections of the colon the results for total collagen content measurement are not affected by aging of the tissue. The results for collagen solubility measurement are more pronounced. There is a significant positive correlation between the acid solubility of collagen and the age of the human subject ($p< 0.001$). Acid insolubility increases rapidly after the age of forty years, in both males and females. This is interesting as colonic diverticulosis is rare before the age of forty. This relationship was observed in all four sections of the colon (see Graphs 4.5-4.8). The relationship was more strongly significant in the sigmoid colon, which is the predominant location for the development of colonic diverticula.

Results also indicate, that tissues from subjects affected by colonic diverticulosis are less soluble in weak acids than age matched healthy colons ($p<0.05$). This would infer that the collagen from those colons with colonic diverticulosis has a higher number of crosslinks than the healthy counterparts. It may mean that aldimine crosslinks have matured to a non acid soluble form.

This may result in increased rigidity of colonic wall tissue.

The results illustrate that collagen from the sigmoid region of the colon is altered in the form of an increase in the amount of acid insoluble collagen in the colonic submucosa. This infers that the number of acid insoluble crosslinks is increased in this colonic tissue. This is a biochemical and structural alteration in the colon collagen. In young, healthy colons the amount of acid insoluble crosslinked collagen is considerably lower. The alteration may be responsible for the development of colonic diverticulosis. It is interesting to note that the amount of acid insoluble collagen, increases both with advancing age of the subject and also as the colon is traversed from right to left. The left side of the colon, and in particular the sigmoid colon, is the predominant location of diverticula in the western population. The increased amount of irreversibly crosslinked collagen in this area could, when coupled with increased intraluminal pressure, result in protrusion through the colon wall in the form of diverticulae. This is a significant result as dietary fibre deficiency was thought by some to be the sole aetiological factor in the development of colonic diverticulosis (Painter N.S., 1975).

The X-ray diffraction data presented in this thesis are worthy of particular attention. These data are singular with respect to this particular tissue and for the study of diverticular disease. Results illustrate that the collagen of the human colon wall is not as ordered as collagen from rat tail tendon (the most ordered form of tendon studied) in either the meridional or equatorial direction. This is illustrated by the smaller number of meridional orders of diffraction which can be observed in human colon collagen, and the reduced number of discrete diffraction peaks in the equatorial direction. Crystalline disorder can easily be identified by the smearing of diffraction peaks over greater areas of the film, indicating imperfections in a crystalline lattice. The immediate

indication from the X-ray diffraction data is that the collagen from the human colon is not as ordered axially, i.e. not as crystalline, as the collagen from rat tail tendon. The diffraction patterns give information on the first 30 orders of diffraction, which is the first information of such a resolution for this tissue.

An important feature of the diffraction patterns is the difference in D period, or repeating unit, between colon collagen and rat tail tendon collagen. The D period for colon collagen is 655Å compared with 670Å for wet rat tail tendon collagen. This would infer a more compacted nature of colon collagen, possibly in the form of a tilt in the molecules with regard to the fibril axis. This theory is further substantiated by the bimodal distribution of density on the equator observed in the high angle diffraction patterns (Plates 4.3, 4.5 and 4.7), which indicates a coiled coil or tilted system.

The collagen from the three groups of tissue examined exhibits a less ordered structure in the aged tissue. The order is further reduced in the aged, diseased tissue, compared with the young, healthy tissue. This could parallel the biochemical changes which have taken place in the form of increased collagen crosslinkage.

The existence of the equatorial peaks as a bimodal distribution parallel to the direction of the meridian is of importance. This indicates that the molecules are tilted through a specific angle with respect to the fibril axis. The only plausible way in which the tilting can occur is through the helical arrangement of collagen molecules around a common axis. Since colon collagen may exist in a helical packing microfibril, this indicates the presence of type III collagen in the microfibrils (Brodsky B. and Eikenberry E.F., 1982).

This thesis has produced the first diffraction data of colonic wall collagen with resolution along the more ordered meridian to 65.5/30 or 2.1nm resolution. The results also illustrate that differences exist between the meridional reflections of those patterns from healthy and pathological tissues. This must correspond to differences between the axial electron density profile of collagen molecules in the healthy and diseased tissues. These changes may be influenced by the presence of excessive crosslinkage, pulling the collagen molecules into a specific and different structure. There appears to be a general decrease in the order (or crystallinity) of the collagen recorded on diffraction patterns associated with increasing age of the subject from which tissue was removed. This observation was more pronounced when diseased tissue was compared to age matched tissues. This may be due to a number of factors. The lateral unit cell of collagen from rat tail tendon expands upon non-enzymatic glycosylation (Tanaka S. *et al*, 1988). This was simultaneous with a disordering of the collagen molecule in the unit cell. A similar observation was made with diabetic and aged tissue (James V.J., McConnell J.F. and Capel M., 1991). A similar mechanism may be occurring in the collagen from the human colon as the order observed in diffraction patterns is different between young, aged and diseased tissues. Biochemical evidence indicates that the collagen is altered by an increase in the amount of acid insoluble collagen (inferred number of crosslinks). The increase in crosslink number may be partly due to the increase in crosslinks of the advanced Maillard product type, since these accumulate in aging tissue. This alteration may give rise to changes in the packing, azimuthal arrangement and electron density of collagen molecules within the tissue. The X-ray diffraction experiments indicate that there are structural differences between the young, healthy colon collagen and the aged, healthy colon collagen. Data presented here serves to substantiate the information gathered regarding the decreased collagen solubilty in the pathological tissues.

So far, no satisfactory method of determining real electron density from mixed fibres of type I and type III collagen has been produced. An approach would be to model the projected electron density of type I and type III collagen. This would require the molecules to be in their correct axial register and the electron density component of each expressed in the correct ratio. A project such as this would require considerable computer programming and is outwith the scope of this thesis.

6.4 The effect of diet on the collagen of the rat colon wall.

The aging of laboratory rat colons does not appear as a prominent feature in the development of colonic diverticula in the experiments presented here. All of the rats were of the same age and only 41.2% of the F.L.F. and 21.1% of the W.L.F. developed colonic diverticulosis. None of the F.H.F. had evidence of colonic diverticulosis or bowel segmentation.

The colonic diverticula were all situated in the mid or left side of the colon (distal colon) which is in accordance with the human study in this thesis. This is in comparison with the original study by Hodgson who noted that the colonic diverticula in the rat were situated in the right side of the colon (proximal colon) (Hodgson I., 1972). The most original observation in the animal experiment in this thesis is the difference in the incidence of colonic diverticulosis between W.L.F. and F.L.F. This can possibly be explained by two events:

(1) Certain types of dietary fibre are known to retain nutrients within their network when in the colon. Perhaps the young on the weaned low fibre diet were not receiving the correct vitamins and minerals during this essential period of development. This would be compounded by the effect of the low fibre diet.

(2)There may be a compound produced by the bacterial fermentation of certain types of dietary fibre in the colon of the parents which may be passed to the young during the development of the colon. This may be protective against the development of colonic diverticulosis.

A study which may be of relevance, has examined the relationship between weaning diets and the condition of the small intestinal wall. This has reported that the feeding of a specific form of dietary fibre influences the condition of the villi of the small intestine. The inference of this study is that there are adaptations occurring in the small intestine of the laboratory rat as early in life as two weeks. This study illustrates that diet affects these adaptations (Cassidy M.M., Fitzpatrick L.R. and Vahouny G.V., 1981). Similar adaptations may also be occurring in the colon of laboratory rats and perhaps humans who are exposed to different types or concentrations of dietary fibre.

The dietary experiment in this thesis produced a series of significant results regarding the structure of rat colonic collagen in relation to dietary composition. One of the most interesting results was that there was a significant difference in the amount of crosslinked collagen as the colon is traversed from right to left ($p<0.001$). This indicates that the collagen, and hence the submucosa of the left colon could be more rigid than the right colon. Interestingly, this observation is paralleled by an increase in the number of collagen fibrils in a defined area and an associated decrease in the fibril diameters in the left side of the colon. This was observed in an electron microscopy study (Thomson H.J. *et al*, 1987a). The significance of this observation is realised, when it is noted that the left side of the colon is the predominant location for the development of colonic diverticula in western populations.

The rats which had developed colonic diverticulosis, had increased levels of acid soluble collagen, as judged by the collagen solubility index, when compared with rats with healthy colons. This suggests that there is another factor involved in the development of diverticula. If the development of colonic diverticula was merely as a consequence of either age or diet then consequently, all of the rats on the low fibre diet which had reached 18 months of age would have developed colonic diverticulosis. This was however not the case, as fewer of the rats in the W.L.F. group developed the condition than in the F.L.F. group. This suggests that this is a novel aetiological factor in the development of colonic diverticulosis.

The most original observation of this study is that there are striking differences between the rats in the F.L.F. and those in the W.L.F., in almost all of the parameters measured. This is the first experiment which has examined the relationship between the effect of maternal diet and the development of colonic diverticula in the laboratory rat. The most striking results produced from this study being the number of tumours and abnormalities which were observed in the rats *post-mortem*. Tumours, or pathological abnormalities were found to be twice as prevalent in the F.L.F. as the W.L.F. who were both fed identical diets and treated in the same manner. This strongly suggests that dietary fibre is an important protective prenatal requirement. The importance of prenatal and infant feeding is currently an area of great interest, with one particular group examining the relationship between maternal diet, socioeconomic conditions and the health of the adult in later life (Barker D.J.P. and Osmond C., 1987), (Barker D.J.P., 1988), (Barker D.J.P., 1989), and (Barker D.J.P. *et al*, 1989). This group have observed that there is a significant relationship between low birth weight and the development of ischaemic heart disease in later life. They have observed that low birth weight babies from economically and socially depressed areas of northern England have a higher incidence of

ischaemic heart disease when they reach the age of 50-60 years. They have correlated the incidence of ischaemic heart disease to poor ante-natal maternal nutrition and perhaps poor feeding in infancy.

The work carried out by Barker is important with respect to this thesis, as all of the animals in this study were of similar birth weights. After the weaning phase and the spurt growth phase, the F.L.F. and W.L.F. group rats grew much more rapidly than the F.H.F. rats which were fed a relatively high fibre diet. This finding was also reported by several groups, some of whom have attributed this to the presence of growth inhibitory n-alkyl resorcinols in wheat (Wieringa G.W., 1967), (Verdeal K. and Lorenz K., 1977). The levels are considered to be nonsignificant for this thesis. At the termination of this experiment, the rats from the two low fibre fed groups (F.L.F. and W.L.F.) were almost twice the weight of the high fibre fed group (F.H.F.). On first inspection this could have been due to the different diets, however there were significant differences between the weights of the F.L.F and the W.L.F at the end of the experiment. Both of these groups were fed identical diets and treated in the same manner. The only difference between the two groups was the maternal diet. The maternal diet was therefore the determining factor in the healthy development of the progeny and not solely the diet on which the progeny were fed. The effect of maternal diet has an important bearing on the health of the young and also the developing adult.

The difference in the composition and consistency of the two diets examined in this study is important. This influences the way in which the diets are transported along the gastrointestinal tract and hence influences the function of the colon. The low fibre diet assumed a putty like consistency when prepared. One would expect the colon to require high intraluminal pressure and energy to propel the small, dry stools (commonly produced by such a diet) along its

length. The high fibre diet in contrast, was dry and pelleted. The fibre would allow a high water holding capacity, producing bulky, soft stools in the colon. These could be more easily propelled along the length of the colon. The two different diets would require very different functions of the colon and this could account for the differences between the F.L.F. and the F.H.F. groups. The differences between the F.L.F. and the W.L.F. rats which were both fed identical diets are not explained by the dietary fibre content. The differences therefore appear to lie in the maternal diet. The difference in the physiology of the colon between humans and laboratory rats cannot be ignored in these experiments. The rat colon acts as a long tube which absorbs water along its entire length, producing pelleted stools along the length of the colon long before the rectum. The human colon does not produce formed stools until much further along its length. In spite of the differences, the laboratory rat remains a viable model for the study of colonic diverticulosis in humans. This is the first evidence which relates the development of a colonic disorder in second generation rats to the diet of the parents.

6.5 Analysis of collagen from both humans and rats. The effects of age and diet.

The dietary fibre theory and the structural theory which have been outlined in this thesis are both important individually in the aetiology of colonic diverticulosis. The dietary fibre theory fits with the fact that colonic diverticulosis can be treated by administering dietary fibre. The incidence of colonic diverticulosis is reduced in those people who consume a vegetarian diet (Gear J.S.S., Ware A. and Fursden P., 1979). The low incidence of colonic diverticulosis in Third World countries also implies that there is a relationship between dietary fibre and the development of colonic diverticulosis. The consumption of dietary fibre is on average, high in these countries compared with industrialised countries (Painter N.S. and Burkitt D.P., 1971). This

thesis corroborates the dietary fibre theory in one aspect. The rats which were maintained on the low fibre diet developed colonic diverticulosis, whereas those fed on the high fibre diet had a healthy bowel.

The structural theory as proposed by Morson in 1963 is the theory which is of most relevance to the work described in this thesis. The structural changes which have been observed in relation to the aging of the tissue are apparently not a function of the diet of that subject, but merely of the aging process. The structural changes to the collagen which were observed in relation to colonic diverticulosis are thought to be a function of the aging process, as the dietary history of the subjects is unknown (Morson B.C., 1963).

It is concluded from this thesis that the two previously presented theories are valid in parallel. The structural changes associated with aging of the colon wall are amplified by high intraluminal pressures produced by a low fibre diet. The proposal for the aetiology of colonic diverticulosis which has come from this thesis, is that there are structural changes in the colonic wall collagen in relation to the aging of that tissue. These changes are amplified in those subjects with colonic diverticulosis, which would indicate that this is linked with an acceleration of the aging process. The changes which take place are, increasing acid stable crosslinking and decreasing solubility of the colon wall collagen. These changes may reduce the natural tensile strength of the colon wall and leave it susceptible to damage. This could mean that colonic diverticulosis is a consequence of aging in the human, and may be aggrevated by a diet which is low in dietary fibre. This is the first structural evidence relating to the development of colonic diverticulosis.

The influence of maternal diet is one of the most significant aspects of this thesis and would benefit from further study. This study illustrates that the age

of the subject is not the sole determining factor in the development of colonic diverticulosis, as all of the rats were of an identical age. This thesis has illustrated that the composition of the maternal diet is of primary importance in the development of a healthy bowel wall. The maternal diet may also aid in the prevention of the development of colonic diverticulosis in later life. This is the first study which has observed structural differences in colonic wall collagen relating to the effect of maternal diet. The significance of these results with regard to future dietary studies is paramount.

This thesis also produces significant evidence that all future dietary studies which examine the structure of the colon, and perhaps other organs of the body, may be affected by the fact that the influence of maternal diet on the development of the young is not considered in the original design of the experiment. From this thesis it is evident that consideration of the effect of maternal diet on the development of the young in any dietary experiment is paramount for the production of meaningful results.

The information regarding the structure of human colonic wall collagen which is contained in this thesis has an important bearing on the future analysis of colonic collagen in healthy tissue, and tissue affected by colonic diverticulosis. It may also have a bearing on the analysis of collagen from tissues affected by related disorders.

In summary this thesis establishes for the first time :

(i) The total collagen content of the human colon wall is not changed by aging. It is also not changed by the position in the colon or the presence of colonic diverticulosis.

(ii) Collagen from the human colon wall becomes increasingly less soluble in

weak acid as the age of the subject increases. This effect is particularly evident after the age of forty years.

(iii) Collagen from the human colon wall becomes increasingly less soluble as the colon is traversed from right to left sides. The sigmoid colon wall contains the highest amount of insoluble collagen.

(iv) Collagen from the human colon wall of subjects affected by colonic diverticulosis is less soluble in weak acid than age matched control colons.

(v) Collagen from the human colon wall is less ordered than collagen from rat tail tendon as exhibited by X-ray diffraction.

(vi) The order of human colon collagen is lower in healthy aged tissue than in healthy young tissue. The order is also significantly lower in aged diseased tissues than in aged healthy tissues.

(vii) The D period of human colon collagen is lower than that of collagen from rat tail tendon. The D period of human colon collagen is 65.5nm compared with 67.0nm in rat tail tendon collagen.

(viii) The X-ray diffraction patterns of human colon collagen exhibit a bimodal distribution, parallel to the equator, indicating a helical microfibril structure.

(ix) The rats in all of the dietary groups consumed a similar amount of food, but the growth patterns were very different. The F.H.F. rats had a much lower average terminal body weight than the W.L.F. and the F.L.F. rats. The W.L.F. rats had a lower average terminal body weight than the F.L.F. rats.

(x) The *post-mortem* results indicated that the F.H.F. rats had a much lower number of tumours or abnormalities of the body organs than both the W.L.F. and the F.L.F.. The W.L.F. rats had a significantly lower number of tumours and abnormalities than the F.L.F. rats.

(xi) The percentage of rats which developed colonic diverticulosis was significantly different between the three groups of rats. The F.H.F. rats had a 0% incidence of colonic diverticulosis. The W.L.F. and the F.L.F. which were both fed the same diet, had incidences of 21.1% and 41.2% respectively.

(xii) The total collagen content of the rat colon wall is not altered by the diet which is fed. It is also not altered by the position along the gastrointestinal tract or the presence of colonic diverticulosis.

(xiii)The amount of acid insoluble collagen is significantly higher in the F.L.F. rats compared with the W.L.F. and the F.H.F. rats.

(xiv) The amount of acid insoluble collagen increases as the colon is traversed from right to left sides.

(xv) The maternal diet appears to affect the health of the young in later life. The maternal diet also appears to affect the probability of the development of colonic diverticulosis. This is illustrated by the fact that the two low fibre fed groups had differences in almost all of the parameters measured, although they were fed the same diet.

The data produced in this thesis have an important bearing on the understanding of the aetiology of colonic diverticulosis, as the effect of aging on the collagen is pronounced.

Bibliography.

Adachi E., Hayashi T. and Hashimoto P. H. (1989). "Immuno electron microscopical evidence that type V collagen is a fibrillar collagen: importance for an aggregating capability of the preparation for reconstituting bandng fibrils". *Matrix* , **9** ; 232-237.

Ahmed M. U., Thorpe S. R. and Baynes J. W. (1986). "Identification of Nε-carboxymethyllysine, a degradation product of fructoselysine in glycated protein". *J. Biol. Chem.*, **261** ; 4889-4894.

Anderson J. W. (1986). "Fibre and Health: an Overview". *J. Gastroent.*, **81** ; 892-897.

Arfwiddson S. (1964). "Pathogenesis of multiple diverticula of the sigmoid colon in diverticular disease". *Acta Chirurgica Scandinavica,* Supplement **342** ; 1-68.

Bailey A. J. and Peach C. M. (1968). "Isolation and structural identification of a labile intermolecular crosslink in collagen". *Biochem. Biophys. Res. Comm.*, **33** ; 812-819.

Barker D. J. P. (1988). "Childhood causes of adult diseases". *Archives of Disease in Childhood*, **63** ; 867-869.

Barker D. J. P. (1989). "Rise and fall of Western diseases". *Nature*, **338** ; 371-372.

Barker D. J. P. and Osmond C. (1987). "Inequalities in health in Britain: specific explanations in three Lancashire towns". *Brit. Med. J.*, **294** ; 794-752.

Barker D. J. P., Osmond C., Winter P. D., Margetts B. and Simmonds S. J. (1989). "Weight in infancy and death from ischaemic heart disease". *Lancet*, 9th September ; 577-580.

Barnard K., Light N. D., Sims T. J. and Bailey A. J. (1987). "Chemistry of the collagen crosslinks. Origin and partial characterisation of a putative crosslink of collagen". *Biochem. J.*, **244** ; 303-309.

Bear R. S. (1944). "X-ray diffraction studies on protein fibres. I. The large fibre - axis period of collagen". *J. Am. Chem. Soc.*, **66** ; 1297-1305.

Birk D. E., Fitch J. M., Babiarz J. P. and Linsenmayer T. F. (1988). "Collagen type I and type V are present in the same fibril in the avian corneal stroma". *J. Cell Biol.*, **106** ; 999-1008.

Boedtker H. and Doty P. (1956). "The native and denatured states of soluble collagen". *J. Am. Chem. Soc.*, **78** ; 4267-4280.

Bond J. H. and Levitt M. D. (1976). "Fate of soluble carbohydrate in the colon of rats and man". *J. Clin. Invest.*, **57** ; 1158-1164.

Bornstein P. (1969). "Comparative sequence studies of rat skin and tendon collagen. II The absence of a short sequence at the amino terminus of the skin $\alpha 1$ chain. *Biochemistry* , **8** ; 63-71.

Bornstein P. and Piez K. A. (1966). "The nature of the intramolecular crosslinks in collagen. The separation and characterisation of peptides from the crosslink region of rat skin collagen. *Biochemistry*, **5** ; 3460-3473.

Bradshaw J. P., Miller A. and Wess T. J. (1989). "Phasing the meridional diffraction pattern of type I collagen using isomorphous derivatives". *J. Mol. Biol.*, **205** ; 685-694.

Brodribb A. J. M. (1980). "Dietary fibre in diverticular disease of the colon". In: Spiller G. A., Kay R. M., eds. Medical aspects of dietary fibre. N.Y. and London, Plenum Medical Book Co. ; 43-46.

Brodribb A. J. M. (1977). "Treatment of symptomatic diverticular disease with a high fibre diet". *Lancet*, **1** ; 664-666.

Brodribb A. J. M. (1981). "Does fibre affect the colon? Recent primate experimental research." In: Baird I. M., Ornstein M. H., eds. Dietary fibre: progress towards the future (Symposium). Manchester, Kellogg Co. of Great Britain ; 21-32.

Brodribb A. J. M. and Humphreys D. M. (1976). "Diverticular disease: three studies, II. Treatment with bran". *Brit. Med. J.*, **i** ; 425-428.

Brodsky B. and Eikenberry E. F. (1982). "Characterisation of fibrous forms of collagen". Structural and contractile proteins Part A : Extracellular matrix. *Methods in Enzymology*, **82** ; 127-184.

Brodsky B. Eikenberry E. F. and Cassidy K. (1980). "An unusual collagen periodicity in skins". *Biochim. Biophys. Acta* **621** ; 162-166.

Buckingham B. and Reiser K. M. (1990). "Relationship between the content of lysyl oxidase dependent crosslinks in skin collagen, nonenzymatic glycosylation and long term complications in type I diabetes mellitus". *J. Clin. Invest.*, **86** ; 1046-1054.

Bunn H. F. and Higgins P. J. (1981). "Reactions of monosaccharides with proteins: possible evolutionary significance". *Science*, **213** ; 222-224.

Burgeson R. E. (1988). "New collagens - new concepts". *Annu. Rev. Cell Biol.*, **4** ; 551-577.

Burkitt D. P. and Trowell H. C. (1975). "Refined carbohydrate foods and disease: the implications of dietary fibre". London: Academic Press.

Burkitt D. P., Walker A. R. P. and Painter N. S. (1972). "Effect of dietary fibre on stools and transit times, and its role in the causation of disease". *Lancet* , **ii** ; 1408-1412.

Burkitt D. P., Clements J. L. and Eaton S. B. (1985). "Prevalence of diverticular disease, hiatus hernia, and pelvic phleboliths in black and white Americans". *Lancet*, **ii** ; 880-881.

Carlson A. J. and Hoelzel F. (1949). "Relation of diet to diverticulosis of the colon in rats." *Gastroenterology* ; **12** ; 108-115.

Cassidy M. M., Fitzpatrick L. R. and Vahouny G. V. (1981). "The effect of fibre in the postweaning diet on nutritional and intestinal morphological indices in the rat." In: Dietary fibre (basic and clinical aspects), Vahouny G. V., Kritchevsky D., eds. ; 229-251.

Cerami A., Vlassara H. and Brownlee M. (1988). "Role of advanced glycosylation products in complications of diabetes". *Diabetes care*, **11** (Suppl. 1) ; 73-79.

Chang K., Uitto J., Rowold E. A., Grant G. A., Kilo C. and Williamson J. R. (1980). "Increased collagen crosslinkages in experimental diabetes. Reversal by ß-aminopropionitrile and D-Penicillamine". *Diabetes*, **29** ; 778-781.

Christmann D., Folkhard W., Knorzer E., Mosler E. and Nemetschek Th. (1989). "X-ray evidence for native twisted collagen fibrils". Synchrotron Radiation Research Report, D.E.S.Y., August 12th.

Cleave T. L., Campbell G. D. and Painter N. S. (1969). "Diabetes, coronary thrombosis and the saccharin disease", second edition. John Wright and Sons Ltd., Bristol.

Cochran W., Crick F. H. C. and Vand V. (1952). "Structure of synthetic polypeptides. (1) Transforms of atoms on a helix". *Acta. Cryst.*, **5** ; 581-586.

Craig A. S. and Parry D. A. D. (1981). "Collagen fibrils of the vertebrate corneal stroma". *J. Ultrastruct. Res.*, **74** ; 232-239.

Cummings J. H., Southgate D. A. Z., Branch W. and James W. (1978). "Colonic response to dietary fibre from carrots, cabbage, apple, bran and guar gum". *Lancet*, **i** ; 5-8.

Daly C. D. and Odland G. F. (1979). "Age related changes in the mechanical properties of human skin". *J. Investigative Dermatology*, **73** ; 84-87.

Davis N. R. and Bailey A. J. (1971). "Chemical synthesis of the reduced form of an intermolecular cross-link of collagen : A re-evaluation of the structure of syndesine". *Biochem Biophys Res Commun.* **45** ; 1416-1422.

Drummond H. (1917). "Sacculi of the large intestine with special reference to their relations to blood vessels of the bowel wall". *Br. J. Surg.*, **4** ; 407-413.

Dunn J. A., McCance D. R., Thorpe S. R., Lyons T. J. and Baynes J. W. (1989). "Age dependent accumulation of Nε-(carboxymethyl)lysine and Nε-(carboxymethyl)hydroxylysine in human skin collagen". *Biochemistry*, **30** ; 1205-1210.

Dyer D. G., Blackledge J. A., Thorpe S. R. and Baynes J. W. (1991). "Formation of pentosidine during nonenzymatic browning of proteins by glucose. Identification of glucose and other carbohydrates as possible precursors of pentosidine *in vivo*". *J. Biol. Chem.*, **266** ; 11654-11660.

Eastwood M. A., Eastwood J. and Ward M. (1976). "Epidemiology of bowel disease". In: Spiller G. A., Amen R. J., eds. Fibre in human nutrition. N.Y. and London, Plenum Press ; 207-240.

Eastwood M.A. and Kay R.M. (1979). "Hypothesis for the action of dietary fibre along the gastrointestinal tract". *Am. J. Clin. Nutr.*, **32** (6) ; 664-667.

Eastwood M. A., Sanderson J., Pocock S. J. (1977). "Variation in the incidence of diverticular disease within the city of Edinburgh". *Gut*, **18** ; 571-574.

Eastwood M. A., Smith A. N., Brydon G. and Pritchard J. (1978a). "Comparison of bran, isphagula and lactulose on colon function in diverticular disease." *Gut* , **19** ; 1144-1147.

Eastwood M. A., Smith A. N., Brydon G. and Pritchard J. (1978b). "Colonic function in patients with diverticular disease." *Lancet,* **i** ; 1181-1182.

Eastwood M. A., Watters D. A. K. and Smith A. N. (1982). "Diverticular disease - is it a motility disorder?". *Clinics in Gastroenterology*, **11** ; 545-561.

Edwards H. C. (1939). "Diverticula and diverticulosis of the intestine." Bristol: John Wright and Sons; London: Simpkin Marshall ; 178-205.

Eikenberry E. F., Childs B., Sheren S. B., Parry D. A., Craig A. S. and Brodsky B. (1984). "Crystalline fibril structure of type II collagen in lamprey notochord sheath". *J. Mol. Biol.*, **176** ; 261-277.

Englyst H. N. and Cummings J. H. (1985). "Measurement of dietary fibre as non-starch polysaccharides." in: Vahouny G. V., Kritchevsky D., eds. Basic and clinical aspects of dietary fibre. New York, NY: Plenum.

Englyst H., Wiggins H. S. and Cummings J. H. (1982). "Determination of the non-starch polysaccharides in plant foods by gas liquid chromatography of constituent sugars as alditol acetates". *Analyst*, **107** ; 307-318.

Epstein E. H. and Munderloh N. H. (1975). "Isolation and characterisationof CNBr peptides of human $[\alpha 1(III)]_3$ collagen and tissue distribution of $[\alpha 1(I)]_2$ and $[\alpha 1(III)]_3$ collagens". *J. Biol. Chem.*, **250** ; 9304-9312.

Eyre D. R. (1987). "Collagen cross-linking amino acids". *Methods in Enzymology* , **144** ; 115-140.

Eyre D. R., Koob T. J. and Van Ness K. P. (1984). "Quantitation of hydroxypyridinium crosslinks in collagen by High Performance Liquid Chromatography". *Anal. Biochem.*, **137** ; 380-388.

Eyre D. R. and Oguchi H. (1980). "Hydroxypyridinium crosslinks of skeletal collagen. Their measurement properties and a proposed pathway of formation." *Biocim. Biophys. Res. Comm.* **92** 403-410.

Eyre D. R., Wu J. and Woods P. E., (1991). "The cartilage collagens: structural and metabolic studies". *J. Rheumatol.*, **18** (Suppl. 27) ; 49-51.

Farmar J. G., Ulrich P. C. and Cerami A. (1988). "Novel pyrroles from sulfite-inhibited Maillard reactions: insight into the mechanism of inhibition". *J. Org. Chem.*, **53** ; 2346-2349.

Fietzek P. P. and Kuhn K. (1975). "Information contained in amino acid sequence of α(1) chain of collagen and its consequences upon formation of triple helix, of fibrils and crosslinks". *Molecular and Cellular Biochemistry*, **8** ; 141-157.

Findlay J. M., Smith A. N. and Mitchell W. D. (1974). "Effects of unprocessed bran on colon function in normal subjects and in diverticular disease". *Lancet*, **i** ; 146-149.

Fisher N., Berry C. S., Fearn T., Gregory J. A. and Hardy J. (1985). "Cereal dietary fibre consumption and diverticular disease: a lifespan study in rats". *Am. J. Clin. Nutr.*, **42** ; 788-804.

Fraser R. D. B., MacRae T. P. and Miller A. (1987). "Molecular packing in type I collagen fibrils". *J. Mol. Biol.*, **193** ; 115-125.

Fraser R.D.B., MacRae T.P., Suzuki E. (1979). "Chain conformation in the collagen molecule". *J. Mol. Biol.*, **129** ; 463-481.

Fraser R.D.B. and Trus B.L. (1986). "Molecular mobility in the gap regions of type I collagen fibrils". *Bioscience Reports*, **6** ; 221-226.

Fujimoto D., Akiba K. and Nakamura N. (1977). "Isolation and characterisation of a fluorescent material from bovine achilles tendon collagen". *Biochem. Biophys. Res. Commun.*, **76** ; 1124-1128.

Fujimoto D., Moriguchi T., Ishida T. and Hayashi H. (1978). "The structure of pyridinoline, a collagen crosslink". *Biochem. Biophys. Res. Commun.*, **84** ; 52-57.

Gabella G. (1987). "The cross-ply arrangement of collagen fibres in the submucosa of the mammalial small intestine". *Cell Tiss. Res.*, **248** ; 491-497.

Gear J. S. S., Ware A. and Fursden P. (1979). "Symptomless diverticular disease: the intake of dietary fibre." *Lancet,* **i** ; 511-514.

Gelman R. A. and Piez K. A. (1980). "Collagen fibril formation in vitro - a quasielastic light scattering study of early stages". *J. Biol. Chem.*, **255** ; 8098-8102.

Hall C. E. and Doty P. (1958). "A comparison between the dimensions of some macromolecules determined by electron microscopy and by physical chemical methods". *J. Am. Chem. Soc.*, **80** ; 1269-1274.

Halsted W. S. (1891). "Intestinal anastomosis". *Bull Johns Hopkins Hospital.*, **II** ; 1-4.

Hamlin C. R., Kohn R. R. and Luschin J. H. (1975). "Apparent accelerated aging of human collagen in diabetes mellitus". *Diabetes*, **24** ; 902-904.

Hamlin C.R., Luschin J.H. and Kohn R.R. (1978). "Partial characterisation of the age related stabilising factor of post-mature human collagen. 1: By the use of bacterial collagenases". *Exp. Geront.*, **13** ; 403-414.

Harding J. J. (1978). "Isolation and partial identification of a crosslinked peptide from bovine corneal collagen". *F.E.B.S. Letters*, **87** ; 251-253.

Hayase F., Nagaraj R. H., Miyata S., Njoroge G. and Monnier V. M. (1989). "Aging of proteins: immunological detection of a glucose derived pyrrole formed during Maillard reaction in vivo". *J. Biol. Chem.*, **263** ; 3758-3764.

Heaton K. W. (1985). "Diet and diverticulosis - New leads". Gut, 26 ; 541-543.

Henkel W. and Glanville R. W. (1982). "Covalent crosslinking between molecules of type I and type III collagen". *Eur. J. Biochem.*, **122** ; 205-213.

Hodge A. J. and Petruska J. A. (1963), in: Aspects of Protein Structure, Ramachandran G.N. ed, London and New York, Academic press ; 289-300.

Hodgson I. (1972). "An animal model to study diverticular disease". *Brit. J. Surg.*, **59** ; 315-317.

Hodgson W. J. B. (1972). "An interim report on the production of colonic diverticula in the rabbit." *Gut*, **13** ; 802-804.

Hofmann H., Voss T., Kuhn K. and Engel J. (1984). "Localisation of flexible sites in thread like molecules from electron micrographs. Comparison of interstitial basement membrane and intima collagens". *J. Mol. Biol.*, **172** ; 325-343.

Hollingsworth D. F. and Greaves J. P. (1967). "Consumption of carbohydrates in the United Kingdom". *Am. J. Clin. Nutr.*, **20** ; 65-72.

Hughes L. E. (1969). "Post mortem survey of diverticular disease of the colon. Part II. The muscular abnormality in the sigmoid colon". *Gut*, **10** ; 344-351.

Hulmes D. J. S., Miller A., Parry D. A. D., Piez K. A. and Woodhead-Galloway J. (1973). "Analysis of the primary structure of collagen for the origins of molecular packing". *J. Mol. Biol.*, **79** ; 137-148.

Hulmes D. J. S., Miller A., White S. W. and Doyle B. B. (1977). "Interpretation of the meridional X-ray diffraction pattern from collagen fibres in terms of the known amino acid sequence". *J. Mol. Biol.*, **110** ; 643-666.

Jacobs L. R. and Schneeman B. O. (1981). "Effects of dietary wheat bran on rat colonic structure and mucosal cell growth". *J. Nutrition,* **111** ; 798-803.

James V. J., McConnell J. F. and Capel M. (1991). "The d-spacing of collagen from mitral valves changes with ageing, but not with collagen type III content". *Biochim. Biophys. Acta*, **1078** ; 19-22.

Jesior J. C., Miller A. and Berthet-Colominas C. (1980). "Crystalline three-dimensional packing is a general characteristic of type I collagen fibrils". *FEBS Letters*, **113** ; 238-240.

Kajava A. V., (1991). "Molecular packing in type I collagen fibrils. A model with neighbouring collagen molecules aligned in axial register". *J. Mol. Biol.*, **218** ; 815-823.

Kalund S., Hogsa B., Grevy C. and Oxlund H. (1990). "Reduced strength of skin in Ehlers Danlos syndrome type III". *Scandinavian J. Rheum.*, **19** ; 67-70.

Keene D. R., Sakai L. Y., Bachinge H .P. and Burgeson R. E. (1987). "Type III collagen can be present on banded collagen fibrils regardless of fibril diameter". *J. Cell Biol.*, **105** ; 2393-2402.

Kent M. J. C., Light N. D. and Bailey A. J. (1985). "Evidence for glucose mediated covalent crosslinking of collagen after glycosylation *in vitro*". *Biochem. J.*, **225** ; 745-752.

Kirwan W. O., Smith A. N., McConnell A. A., Mitchell W. D. and Eastwood M. A. (1974). "Action of different bran preparations on colonic function". *Brit. Med. J.*, **iv** ; 187-189.

Kivirikko K. I., Laitinen O. and Prockup D. J. (1967). "Modifications of a specific assay for hydroxyproline in urine". *Anal. Biochem.*, **19** ; 249-255.

Kocour E. J. (1937). "Diverticulosis of the colon ; its incidence in 7000 consecutive autopsies with reference to its complications". *Am. J. Surg.*, **37** ; 433-436.

Kodama T., Freeman M., Rohrer L., Zabrecky J., Matsudaira P. and Krieger M. (1990). " Type I macrophage scavanger receptor contains alpha helical and collagen like coiled coils". *Nature*, **343** ; 531-535.

Komoro T. (1988). "The lattice arrangement of the collagen fibres in the submucosa of the rat small intestine : scanning electron microscopy". *Cell and Tissue research*, **251** ; 117-121.

Last J. A., Armstrong L. G. and Reiser K. M., (1990). "Biosynthesis of collagen crosslinks". *Int. J. Biochem.*, **22** ; 559-564.

Ledl F. and Schleicher E. (1990). "New aspects of the Maillard reaction in foods and in the human body". *Angew. Chem. Int. Ed. Engl.*, **29** ; 565-594.

Lubbock D. M., Thomson W., and Garry R. C. (1937). "Epithelial overgrowths and diverticula in the gut of rats fed on a human diet." *Br. Med. J.*, **1** ; 1252-1253.

Maillard L. C. (1912). "Action des acidés amines sur les sucres: formation des mélanoides par voie méthodique". *C. R. Acad. Sci.*, **154** ; 66-68.

Mandelkow E., Mandelkow E. M. and Bordas J. (1981). "Stages of tubulin assembly studied by time resolved X-ray scattering". *Eur. J. Cell Biol.*, **24** ; 336-341.

Manousos O. N. (1989). "Diverticular disease of the colon". *Digestive Diseases*, **7** (2) ; 86-103.

Manousos O. N., Truelove S. C. and Lumsden K. (1967). "Prevalence of colonic diverticulosis in the general population of the Oxford area". *Lancet* , **iii** ; 762-774.

Manousos O. N., Day N. E. and Tzonou A. (1985). "Diet and other factors in the aetiology of diverticulosis: an epidemiologic study in Greece". *Gut*, **26** ; 544-549.

Marchini M., Morocutti M., Ruggeri A., Koch M.H.J., Bigi A. and Roveri N. (1986). "Differences in the fibril structure of corneal and tendon collagen. An electron microscopy and X-ray diffraction investigation". *Connective Tis. Res.*, **15** ; 269-281.

Maurice D.M. (1957). "The structure and transparency of the cornea". *J. Physiol.*, **136** ; 263-286.

Mechanic G. L., Gallop P.M. and Tanzer M.L. (1971). "Nature of crosslinking in collagens from mineralised tissues". *Biochem. Biophys. Res. Commun.*, **45** ; 644-653.

Meek K.M., Elliott G.F., Hughes R.A. and Nave C. (1982). "The axial electron density in collagen fibrils from human corneal stroma". *Curr. Eye Research*, **1** ; 281-284.

Mendeloff A. I. (1986). "Thoughts on the epidemiology of diverticular disease". *Clinics in Gastroenterology*, **15** ; 855-877.

Mendeloff A. I. (1987). "Dietary fibre and gastrointestinal disease". *Am. J. Clin. Nutr.*, **45** ; 1267-1270.

Miller A. (1982). "Molecular packing in collagen fibrils". *T.I.B.S.*, **7** ; 13-18.

Miller E. J. (1971). "Collagen crosslinking - identification of two cyanogen bromide peptides containing sites of intermolecular crosslink formation in cartilage collagen". *Biochem. Biophys. Res. Comm.*, **45** ; 444-451.

Miller E. J. and Gay S. (1987). "The collagens : an overview and update". *Methods in Enzymology*, **144** ; 3-41.

Miller E. J. and Matukas V. J. (1969). "Chick cartilage collagen. A new type of alpha1 chain not present in bone or skin". *Proc. Nat. Acad. Sci. U.S.A.*, **64** ; 1264-1268.

Morgan M. N. and Ellis H. (1969). "Diverticulosis of the colon and diet." *Br. Med. J.*, **2** ; 53-54.

Morgan P. H., Jacobs H. G., Segrest J. P.and Cunningham L. W. (1970). " A comparative study of glycopeptides derived from selected vertebrate collagens". *J. Biol. Chem.*, **245** ; 5042-5048.

Moriguchi T. and Fujimoto D. (1978). "Age related changes in the content of the collagen crosslink, pyridinoline". *J. Biochem.*, **84** ; 933-935.

Morson B. C. (1963). "The muscle abnormality in diverticular disease of the sigmoid colon". *Brit. J. Radiology*, **36** ; 385-392.

Nagahashi M., Yamazaki O., Ohi G., Kai I., Suzuki H., Hayakawa K., Nagasako K. and Kimura K. (1985). "Dietary fibre intake and diverticular disease of the colon - a case controlled study". *Nippon Eiseigaku Zasshi*, **40** ; 781-788.

Nakamura Y. (1987). "Structure of type I collagen dimers". *Int. J. Biol. Macromol.*, **9** ; 281-290.

Njoroge F. G., Fernandes A. A. and Monnier V. M. (1988). "Mechanism of formation of the putative advanced glycosylation endproduct and protein crosslink 2-(2-furoyl)-4(5)-(2-furanyl)-1-H-imidazole". *J. Biol. Chem.*, **263** ; 1646-1652.

Nold J. G., Kang A. H. and Gross J. (1970). "Collagen molecules: distribution of alpha chains". *Science* , **170** ; 1096-1098.

North A. C. T. Cowan P. M. and Randall J. T. (1954). "Structural units in collagen fibrils ". *Nature*, **174** ; 1142-1143.

Ohi G., Minowa K. and Oyama T. (1983). "Changes in dietary fibre intake among Japanese in the 20th century : a relationship to the prevalence of diverticular disease". *Am. J. Clin. Nutr.*, **38** ; 115-121.

Okuyama K., Arnott S., Takayama M. and Kakudo M. (1981). "Crystal and molecular structure of a collagen-like polypeptide (PRO-PRO-GLY)10". *J. Mol. Biol.*, **152** ; 427-443.

Ornstein M. H., Littlewood E. R., Baird I. M., Fowler J., North W. R. S. and Cox A. G. (1981). "Are fibre supplements really necessary in diverticular disease of the colon. A controlled clinical trial". *Brit. Med. J.*, **i** ; 1353-1356.

Painter N. S., Almeida A. Z. and Colebourne K. W. (1972). "Unprocessed bran in treatment of diverticular disease of the colon". *Brit. Med. J.*, **2** ; 137-140.

Painter N. S. and Truelove S. C. (1964). "The intraluminal patterns in diverticulosis of the colon. Part 1 Resting patterns of pressure." *Gut*, **5** ; 201-213.

Painter N. S., Truelove S. C., Ardran G. M. and Tuckey M. (1965). "Segmentation and the localisation of intraluminal pressures in the human colon, with particular reference to the pathogenesis of colonic diverticula". *Gastroenterology,* **49** ; 169-177.

Painter N. S. (1975). "Diverticular disease of the colon". William Heinemann Medical Books, London.

Painter N. S. and Burkitt D. P. (1971). "Diverticular disease of the colon: A deficiency disease of Western civilisation". *Brit. Med. J.*, **2** ; 450-454.

Painter N. S. and Burkitt D. P. (1975). "Diverticular disease of the colon; a 20th century problem". *Clin. Gastroenterol.*, **4** ; 3-21.

Parry D. A. D. (1988). "The molecular and fibrillar structure of collagen and its relationship to the mechanical properties of connective tissue". *Biophys. Chem.*, **29** ; 195-209.

Parry D. A. D., Barnes G. R. and Craig A. S. (1978a). "Tendon and ligament from the horse : an ultrastructural study of collagen fibrils and elastic fibres as a function of age". *Proc. R. Soc. Lond.*, **203** ; 293-303.

Parry D. A. D., Barnes G. R. and Craig A. S. (1978b). "A comparison of the size distribution of collagen fibrils in connective tissue as a function of age and a possible relation between fibril size, distribution and mechanical properties". *Proc. R. Soc. Lond.*, **203** ; 305-321.

Paul A. A. and Southgate D. A. T. (1978). "McCance and Widdowson's the composition of foods" 4th ed. London, U.K.: H. M. S. O., M. R. C. Spec. Report 297.

Perejda A. J., Zaragoza E. J., Eriksen E. and Uitto J., (1984). "Nonenzymatic glucosylation of lysyl and hydroxylysyl residues in type I and type II collagens". *Collagen Rel. Res.*, **4** ; 427-439.

Piez K. A., Eigner E. A. and Lewis M. S. (1963). "The chromatographic separation and amino acid composition of the subunits of several collagens". *Biochemistry*, **2** ; 58-65.

Pongor S., Ulrich P. C., Bencsath F. A. and Cerami A. (1984). "Aging of proteins: Isolation and identification of a fluorescent chromophore from the reaction of polypeptides with glucose". *Proc. Natl. Acad. Sci. U.S.A.*, **81** ; 2684-2688.

Ramachandran G. N. (1988). "Stereochemistry of collagen". *Int. J. Pept. Protein Res.*, **31** ; 1-16.

Ramachandran G. N. and Kartha K. (1954). "Structure of collagen". *Nature* , **174** ; 269-270.

Reid K.B.M. (1974). "A collagen-like amino acid sequence in a polypeptide chain of human C1q (a subcomponent of the first component of complement)". *Biochem. J.*, **141** ; 189-203.

Report of the Royal College of Physicians (1980). "Medical aspects of dietary fibre". Turnbridge Wells, Pitman Medical.

van der Rest M. and Garrone R. (1991)."Collagen family of proteins". *FASEB J.*, **5** ; 2814-2823.

Ricard-Blum S. and Ville G. (1989). "Collagen crosslinking". *Int. J. Biochem.*, **21** ; 1185-1189.

Rich A. and Crick F. H. C. (1955). "Structure of collagen". *Nature* , **176** ; 915-916.

Robins S. P. and Bailey A. J. (1972). "Age related changes in collagen. The identification of reducible lysine carbohydrate condensation products". *Biochem. Biophys. Res. Comm.*, **48** ; 76-84.

Robins S. P. and Bailey A. J. (1975). "The chemistry of the collagen crosslinks. The mechanism of stabilisation of the reducible intermediate crosslinks". *Biochem. J.*, **149** ; 381-383.

Robins S. P. and Duncan A. (1983). "Crosslinking of collagen: Location of pyridinoline in bovine articular cartilage at two sites of the molecule". *Biochem. J.*, **215** ; 175-182.

Robins S. P., Shimokomaki M. and Bailey A. J. (1973). "The chemistry of the collagen crosslinks. Age related changes in the reducible components of intact bovine collagen fibres". *Biochem. J.*, **131** ; 771-780.

Schmitt F. O., Hall C. E. and Jakus M. A. (1942). "Investigation of collagen by electron microscopy". *J. Cell. Comp. Physiol.*, **20** ; 11-33.

Schnider S. L. and Kohn R. R. (1981). "Effects of age and diabetes mellitus on the solubility and nonenzymatic glucosylation of human skin collagen". *J. Clin. Invest.*, **67** ; 1630-1635.

Schnider S. L. and Kohn R. R. (1982). "Effects of age and diabetes mellitus on the solubility of collagen from human skin, tracheal cartilage and dura mater". *Exp. Gerontol.*, **17** ; 185-194.

Segal I., Soloman A. and Hunt J. A. (1977). "Emergence of diverticular disease in the urban South African black". *Gastroenterology* , **72** ; 215-219.

Sell D. R. and Monnier V. M., (1989). "Isolation, purification and partial characterisation of novel fluorophores from aging human insoluble collagen-rich tissue". *Connective Tissue Research*, **19** ; 77-92.

Sell D. R. and Monnier V. M. (1990). "End stage renal disease and diabetes catalyse the formation of a pentose derived crosslink from aging human collagen". *J. Clin. Invest.*, **85** ; 380-384.

Slack W. W. (1966). " Bowel muscle in diverticular disease". *Gut*, **7** ; 668-670.

Smith A. N., Drummond E. and Eastwood M. A. (1981). "The effect of coarse and fine Canadian red spring wheat and French soft wheat bran on colonic motility in patients with diverticular disease." *Am. J. Clin. Nutr.*, **34** ; 2460-2463.

Smith A. N., Shepherd J. and Eastwood M. A. (1981). "Pressure changes after balloon distension of the colon wall in diverticular disease". *Gut,* **22** ; 841-844.

Smith J.W. (1968). "Molecular pattern in native collagen". *Nature*, **219**; 157-158.

Spiller G. A., Chernoff M. C. and Shipley E. A. (1977). "Can faecal weight be used to establish a recommended intake of dietary fibre". *Am. J. Clin. Nutr.*, **30** ; 659-661.

Srivistava G. S., Smith A. N., Painter N. S. (1976). "Sterculia bulk-forming agent with smooth relaxant versus bran in diverticular disease". *Br. Med. J.*, **i** ; 315-318.

Stemmerman G. N. (1970). "Patterns of disease among Japanese living in Hawaii". *Arch. Envir. Health*, **20** ; 266-273.

Sugihara K., Muto T., Morioka Y., Asano A. and Yamamoto T. (1984). "Diverticular disease of the colon in Japan". *Dis. Colon Rectum*, **27**; 531-539.

Tanaka S., Avigad G., Brodsky B. and Eikenberry E. F. (1988). "Glycation induces expansion of the molecular packing of collagen". *J. Mol. Biol.*, **203** ; 495-505.

Tanzer M. L. (1973). "Crosslinking of collagen. Endogenous aldehydes in collagen react in several ways to form a variety of unique covalent crosslinks". *Science*, **180** ; 561-566.

Thompson W. G., Patel D. G., Tao N. and Nair R. C. (1982). "Does uncomplicated diverticular disease produce symptoms?". *Digestive Diseases and Sciences*, **27** ; 605-608.

Thomson H. J., Busuttil A., Eastwood M. A., Smith A. N. and Elton R. A., (1987a). "Submucosal collagen changes in the normal colon and in diverticular disease". *Int. J. Colorect. Dis.*, **2** ; 208-213.

Thomson H. J., Busuttil A., Eastwood M. A., Smith A. N., Elton R. A., (1987b). "The submucosa of the human colon". *J. Ultrastruct. Res.*, **96** ; 22-30.

Thornton J. R., Dryden A., Kelleher J. and Losowsky M. S. (1986). "Does super efficient starch absorption promote diverticular disease". *Br. Med. J.* [Clin. Res.], **292** ; 1708-1710.

Tomlin S. G. and Worthington C. R. (1956). "Low angle X-ray diffraction patterns of collagen". *Proc. R. Soc.*, Ser. A, **235** ; 189-201.

Verdeal K. and Lorenz K. (1977). "Alkylresorcinols in wheat, rye and triticale." *Cereal Chem.*, **54** ; 475-483.

Vuorio E. and de Crombrugghe B. (1990). " The family of collagen genes". *Annu. Rev. Biochem.*, **59** ; 837-872.

Watt J. and Marcus R. (1964). "The pathology of diverticulosis of the intertaenial area of the pelvic colon." *J. Path. Bact.*, **88** ; 97-103.

Watters D. A. K. (1983). "The mechanical properties of the bowel in health and disease". Ch.M. Thesis, University of Edinburgh.

Watters D. A. K. and Smith A. N. (1990). "Strength of the colon wall in diverticular disease". *Br. J. Surg.*, **77** ; 257-259.

Watters D. A. K., Smith A. N., Eastwood M. A., Anderson K. C., Elton R. A. and Mugerwa J. W. (1985). "Mechanical properties of the colon: comparison of the features of the African and European colon *in vitro* ". *Gut* , **26** ; 384-392.

Weinreich J. and Andersen D. (1976). "Intraluminal pressure in the sigmoid colon. II. Patients with sigmoid diverticula and related conditions." *Scandinavian J. Gastroent.*, **11** ; 581-586.

Weiss J. B. and Jayson M. I. V. eds (1982). "Collagen in health and disease". Edinburgh, Churchill Livingstone.

Wess T. J., Miller A. and Bradshaw J. P. (1990). "Crosslinkage sites in type I collagen fibrils studied by neutron diffraction". *J. Mol. Biol.*, **213** ; 1-5.

Whiteway J. and Morson B. C. (1985 a). "Pathology of the aging - diverticular disease". *Clinics in Gastroenterology*, **14** ; 829-846.

Whiteway J. and Morson B. C. (1985 b). "Elastosis in diverticular disease of the sigmoid colon". *Gut*, **26** ; 258-266.

Wieringa G. W. (1967). "On the occurrence of growth inhibiting substances in rye". Wageningen, Publication no. 156, Institute for storage and processing of agricultural produce.

Wyman J. B., Heaton K. W., Manning A. P. and Wicks A. C. B. (1976). "The effect on intestinal transit and faeces of raw and cooked bran in different doses". *Am. J. Clin. Nutr.*, **29** ; 1474-1479.

Yamauchi M., Katz P. Z. and Mechanic G. L. (1986). "Intermolecular crosslinking and stereospecific molecular packing in type I collagen fibrils of the periodontal ligament". *Biochemistry*, **25** ; 4907-4913.

Yamauchi M., Katz E. P., K. Otsubo, K. Teraoka and G. L. Mechanic, (1989). "Crosslinking and stereospecific structure of collagen in mineralised and nonmineralised skeletal tissues". *Connective Tissue Research*, **21** ; 159-169.

Yamauchi M., London R. E., Guenat C., Hashimoto F. and Mechanic G. L. (1987). "Structure and formation of a stable histidine based trifunctional crosslink in skin collagen". *J. Biol. Chem.*, **262**; 1428-1434.